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Journal of the American Association of Clinical Chemists

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Journal of the American Association of Clinical Chemists

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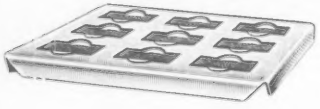

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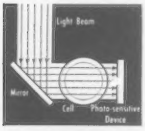

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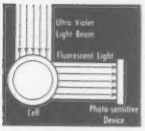

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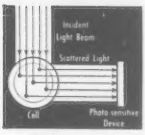

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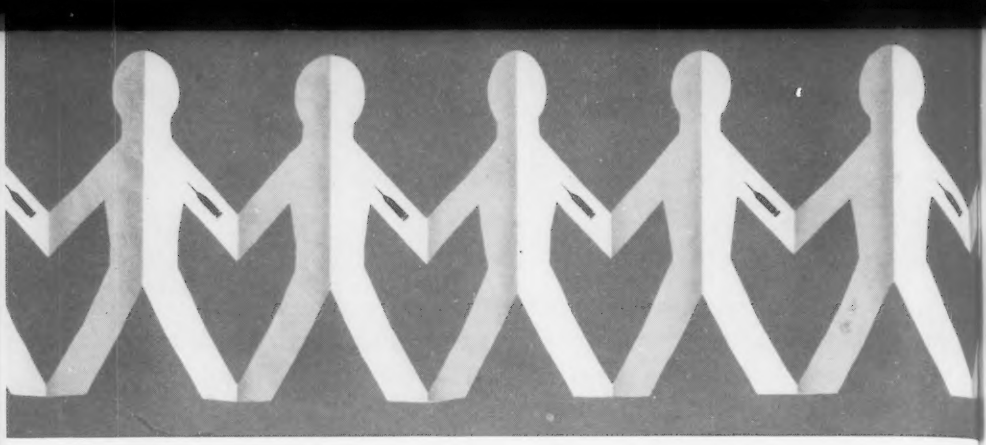

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CLINICAL CHEMISTRY

Journal of the American Association of Clinical Chemists

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On the Determination of "Pancreatitis Lipase" in Serum

Richard J. Henry, Charles Sobel, and Sam Berkman

In spite of a rather voluminous literature on lipolytic enzymes over the past 50 years there appears to be a widespread misunderstanding about the determination of "lipase" in serum as a diagnostic test for acute pancreatitis.

The use of olive oil as a substrate for measuring lipolytic activity can be traced back into the nineteenth century. The extent of hydrolysis was determined by titration of the released fatty acids. In 1911 Rona and Michaelis (1) used tributyrin as substrate, determining the degree of hydrolysis by stalagmometric measurements; i.e. the number of drops from a capillary tube per unit time, which is increased by the decrease in surface tension resulting from hydrolysis. Willstätter and co-workers in 1923 used both tributyrin (2) and olive oil (3) in their studies on pancreatic extracts.

Cherry and Crandall in 1932 (4) applied the olive-oil procedure to serum and found that, following pancreatic duct ligation in dogs, "lipase" increased if olive oil was used as substrate but not when either ethyl butyrate or tributyrin was used. Comfort and Osterberg (5, 6, 7) using the olive-oil technic of Cherry and Crandall found increased values in 83 of 84 cases of pancreatitis. That there is increased serum lipase activity in this disease, when this technic is used, has been confirmed by others (8, 9). Increased values may also occur in carcinoma of the pancreas (10) and hepatic disease (6, 10).

The hydrolysis of tributyrin by serum has been shown to be due to a pseudocholinesterase (9, 11, 12). Goldstein and Roe (13) reported that in cats poisoned by chloroform, primarily a liver poison, there was decreased serum amylase and olive oil "lipase" but increased hydrolysis of ethyl butyrate and tributyrin.

From the Bio-Science Laboratories, 2231 S. Carmelina Ave., Los Angeles, Calif.
Received for publication August 8, 1956.

The terminology which is more or less accepted today defines esterases as enzymes which split organic esters of low molecular weight, and lipases as enzymes which split glycerol esters of fatty acids of high molecular weight, i.e., fats. This division is relative only. Esterases have generally been regarded as originating mainly in the liver, while lipases come primarily from the pancreas. Many papers have been published on studies of the kinetics of the lipolytic activity in pancreatic extracts and there is no question that such extracts do split tributyrin as well as many other lower fatty-acid esters. Several European investigators, using stalagmometric measurements with tributyrin as substrate [refs. cited in (14)], have claimed that pancreatic lipase in serum does hydrolyze tributyrin when calcium oleate is present. Lagerlöf (15) reported increased values in 17 of 18 cases of acute pancreatitis by such a method.

By 1948 the validity of the olive-oil method as a diagnostic test for pancreatitis was well established. Because it involved certain technical difficulties laboratories were receptive to a new test that could be run in a shorter period of time than 24 hours. In 1948 Goldstein, Epstein, and Roe (16) modified a method using tributyrin previously introduced by them in 1943 (17). This method is still used in many laboratories despite the lack of any clinical evidence of its validity as a diagnostic test. In fact it was published as an approved procedure for lipase in *Standard Methods of Clinical Chemistry* (18). Subsequent to this publication it was reported (9) that no increase in "tributyrinase" was detected in 8 cases of acute pancreatitis.

The work presented herein was undertaken at the suggestion of the Editorial Committee of *Standard Methods* of the American Association of Clinical Chemists with the view of replacing the tributyrinase method appearing in *Standard Methods of Clinical Chemistry, Vol. 1*, with a method which would be a valid diagnostic test in acute pancreatitis.

EXPERIMENTAL

Substrates

A brief search was made in the hope that a substrate could be found which could be easily prepared, relatively stable and, above all, would be hydrolyzed more rapidly than olive oil, so that a technic could be evolved requiring a shorter period of incubation. The lipolytic activity of pancreatin (Armour Laboratories, Chicago, Lot No. M 30801) and sera from cases of acute pancreatitis were compared on olive oil by the method of Cherry and Crandall (4), on tributyrin by the method of Goldstein, Epstein, and Roe (16), on glycerol monostearate, and on evaporated milk.

In the cases of milk and glycerol monostearate the Cherry and Crandall technic was followed, substituting milk or a 1% emulsion of glycerol monostearate for the olive-oil emulsion. The milk was brought to *p*H 7.0 before use. The emulsion of glycerol monostearate was prepared by adding the ester (cosmetic grade, Process Chemical Co., Los Angeles) to boiling water slowly with stirring. Myverol® (Type 18-05, control no. S 11789, Distillation Products Industries, Rochester, N. Y.), a purer sample of the ester, was also used, although emulsification was much more difficult. Typical results, confirmed repeatedly, are shown in Table 1. It is seen that pancreatin hydrolyzed olive oil, the fat in milk, glycerol monostearate, and tributyrin. The lipolytic enzyme in the sera from patients with acute pancreatitis did not hydrolyze milk fat or glycerol monostearate. There was some hydrolysis of tributyrin, but no more than that observed with normal sera. (Normal limits given by Goldstein *et al.* (16) are 81 to 246 units.) Because of the importance of this observation, sera from 14 cases of acute pancreatitis were studied by the method of Goldstein *et al.* and by the modified olive oil method of Cherry and Crandall as described later in this communication. The results are shown in Table 2. In not one case was there an elevation of "tributyrinase." This confirms the report of Bunch and Emerson (9). Actually, the levels are frequently lower than normal.

These experiments indicated a very high specificity of the enzyme or enzymes in sera from cases of acute pancreatitis for triglycerides, and no further search was made for an alternative substrate.

Study of the Olive Oil Method

As a starting point, the technic of Cherry and Crandall (4) was followed.

Titration Curves and Indicators

A sample of oleic acid (Lot no. 288, "purity greater than 99%" Mann Research Laboratories, Inc., N. Y. 6) was weighed and dissolved in ethanol to give a concentration of 0.0658N, assuming it to be 100% pure.

Table 1. The Activity of Pancreatin and "Pancreatitis Lipase" as Measured by Various Substrates

	Cherry Crandall units			*Goldstein et al. units*
	Olive oil	*Evaporated milk*	*Glycerol monostearate*	*Tributyrin*
Pancreatin, 1% solution	7.0	2.5	2.1	200
Serum from acute pancreatitis	7.7	0	0	80

Table 2. Comparative Results Obtained with Sera from Patients with Acute Pancreatitis by the Olive-Oil Method and the Method of Goldstein *et al.*

	Units[a]	
Patient. no.	*Olive oil*	*Tributyrin*
1	0.5	90
2	2.9	65
3	3.2	50
4	0.5	75
5	7.7	80
6	7.8	130
7	4.4	95
8	6.0	184
9	8.8	48
10	7.3	140
11	16.3	116
12	4.3	84
13	10.1	28
14	5.8	100

[a] Normal ranges: up to 1.5 for olive oil and 81 to 246 for tributyrin

Titration of this solution with 0.0503N aqueous NaOH, using either thymolphthalein or thymol blue as indicator, indicated it to be 0.0651N, or 99% of theoretical. Three milliliters of the oleic acid solution were thus equivalent to 3.88 ml. of the standard alkali employed. To obtain a titration curve of the oleic acid in the environment existing in the Cherry-Crandall technic, the first step taken was the titration of a mixture of 3.00 ml. of the ethanolic solution of oleic acid, 6.0 ml. water, and 0.50 ml. M/15 phosphate buffer *p*H 7.0. Due to the very limited solubility of oleic acid in water, most of the alkali required for titration of the oleic acid was added before dilution with buffer and water and the remaining oleic acid was titrated potentiometrically. Fig. 1 *A* shows this titration curve, as well as the titration curve of a blank composed of a mixture of 6.0 ml. water, 0.50 ml. buffer, and 3.0 ml. ethanol, and the difference between the two curves which is indicated by the broken line. When 3.88 ml. alkali had been added, the amount required to react stoichiometrically with the oleic acid, the *p*H was 10.5.

The experiment was repeated, substituting a mixture of 3.0 ml. water, 2.0 ml. olive-oil emulsion, and 1.0 ml. serum for the 6.0 ml. of water. The titration curves for blank, oleic acid, and their difference are shown in Fig. 1 *B*. Above a *p*H of about 9 the protein is being titrated. The protein, however, is present in equal amount in both blank and the mixture of blank and oleic acid and the stoichiometric end point for titration of the oleic acid still occurs at *p*H 10.5.

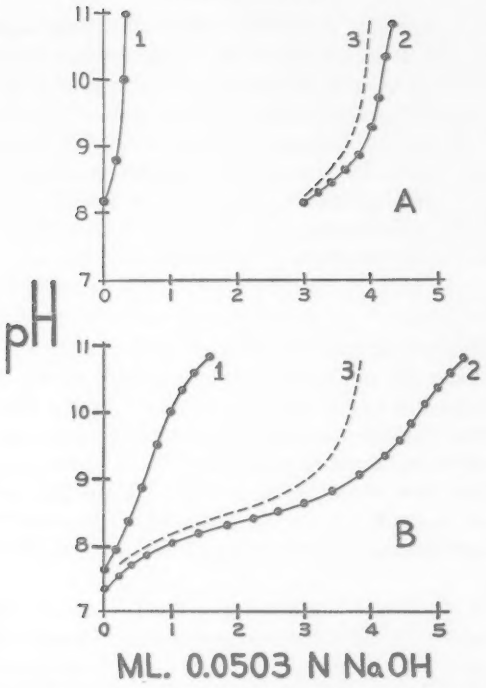

Fig. 1. Titration curves of oleic acid. Model G Beckman *p*H meter used. *A*, without serum; *B*, with serum. Curve 1 = blank; Curve 2 = blank + oleic acid; Curve 3 = difference between Curves 1 and 2.

From the inspection of Fig. 1 *A* it would appear from extrapolation that the *p*K of oleic acid in a water-ethanol mixture is about 8. Fig. 1 *B* indicates that the addition of serum and olive oil emulsion raises the *p*K to about 8.4. Titration curves of the acid produced from olive-oil emulsion by action of sera from patients with acute pancreatitis are quite similar to that of Fig. 1 *B* with estimated *p*K's of about 8.2 to 8.4.

Titration to the equivalence point, *p*H 10.5, by use of a *p*H meter, would be the most accurate technic, but also the most laborious. If phenolphthalein is used as indicator, as in the original Cherry-Crandall procedure, only about 70 per cent of the fatty acids released are titrated since the mixture turns pink at *p*H 8.8. Thymolphthalein, the indicator used by Willstätter and coworkers (3), gives a very pale blue at *p*H 10.1, becomes distinctly blue at *p*H 10.5, and a deep blue at *p*H 10.8. The endpoint with thymolphthalein is not very sharp, but it is estimated that if

one titrates to a "distinct" blue, the error is less than 5 per cent of the total titration. This is considered adequate for routine determinations.

The purpose of the addition of an organic solvent such as alcohol or an alcohol-ether mixture prior to titration has been: (1) to stop enzymatic activity, and (2) to put the free fatty acids into solution for titration. If titration is not delayed the first object is of no consequence. As for the second purpose, identical results were obtained with and without the addition of the alcohol, although the *p*K of the fatty acids appeared to be somewhat lower in the presence of the alcohol.

Blanks

The blank titration includes the alkali required by the buffer, emulsion, and serum to raise the *p*H to the end point used. Serum inactivated at 70° for 5 minutes and added to the blank tube at the beginning of the incubation period has been proposed in some textbooks [e.g. (19)]. It was found safer and more convenient to follow one of two alternative procedures: (1) add the serum to the incubated blank immediately before titration, or (2) add 1.0 ml. serum to 3.0 ml. water and 0.5 ml. buffer, inactivate in boiling water for 5 to 10 minutes, cool, add olive-oil emulsion, and incubate.

It was found in several hundred experiments that the blank titration for any particular olive-oil emulsion remained practically constant. This confirms the claim of Cherry and Crandall. The purpose of running a blank, therefore, is to check on technic and reagents, and one should be run with each set of determinations. It is not necessary to run a blank with each serum and, in fact, if one unknown is run and the quantity of sample is limited, a blank employing another serum can be used.

Substrate Emulsion

In the original Cherry-Crandall technic, equal amounts of olive oil and 5 per cent gum acacia in water containing 0.2% sodium benzoate were emulsified. In a search for a more stable emulsion, emulsification procedures using Carbapol 934 (20), bile salts (21), and polyvinyl alcohol (22) have been proposed. Cherry-Crandall mixtures, emulsified either by passing through a hand homogenizer or in a Waring Blendor for 15 minutes, were compared with the method of preparation employing Carbapol 934. Both of the Cherry-Crandall emulsions were stable for 2 weeks in the refrigerator and then a separation into two phases occurred. The Carbapol mixture was stable for less than one week and was unusable after separation into two phases occurred. Originally, results with these

three emulsions were essentially the same, but upon breakdown, the Carbapol emulsion gave significantly lower results. On the other hand, the Cherry-Crandall emulsions were usable for at least two more weeks after separation when shaken before use. Possibly this may be due to the fact that the Carbapol 934 emulsion separated into a top oil phase and a bottom oil-in-water emulsion, whereas the Cherry-Crandall emulsion separated into a top oil-in-water emulsion and a bottom water phase.

Throughout this work, Old Monk Olive Oil (Old Monk Co., N. Y.) was used. Although identical results were obtained with U. S. P. Olive Oil, the Old Monk product was found easier to emulsify, requiring a maximum of five passages through a hand homogenizer to produce a smooth, white emulsion.

Time of Incubation

Figure 2 gives the degree of hydrolysis as a function of the time of incubation for 5 sera. The original Cherry-Crandall technic called for a 24-hour incubation period. The shape of the curve relating hydrolysis and time deviates sufficiently from zero order so that there is little increase in hydrolysis after 16 hours. Practically, it may be more convenient

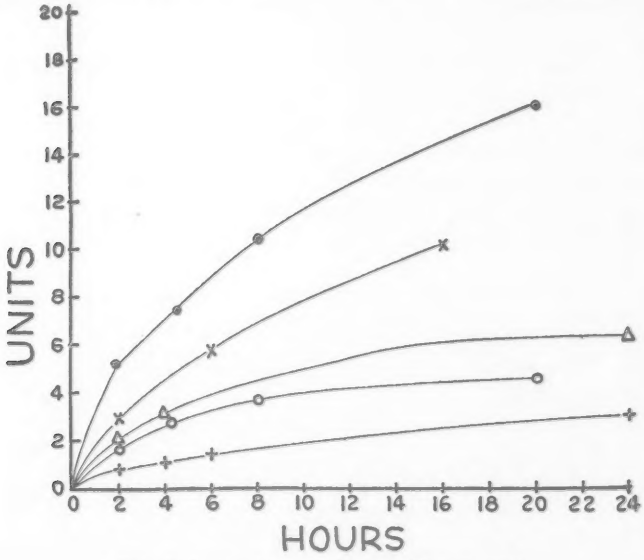

Fig. 2. Time of incubation (5 different sera).

for the laboratory to set up, late in the afternoon, all samples received during the day and titrate them the next morning. A 4-hour incubation time has been suggested (9). In the case of the 5 samples shown in Fig. 2, the values at 4 hours would average 50 per cent (range of 44 to 58 per cent) of those obtained at 16 hours. Specimens received in the morning thus could be reported out the same day, but the advantage would be lost for specimens received in late afternoon. Use of two different periods of incubation may lead to confusion.

Temperature of Incubation

Figure 3 gives the results obtained with two pancreatitis sera at different temperatures. The optimal temperature is at about 40°. It would be more convenient for most laboratories, however, to incubate at 37°.

Buffers and pH

No extensive study of the effect of varying the *p*H and the buffer was made on the lipolytic action of sera from acute pancreatitis. The initial *p*H of a mixture of 3.0 ml. water, 2.0 ml. emulsion, 0.5 ml. M/15 phosphate buffer (*p*H 7.0), and 1.0 ml. serum was 7.2. After incubation, the final *p*H's, without the addition of alcohol, of mixtures using sera con-

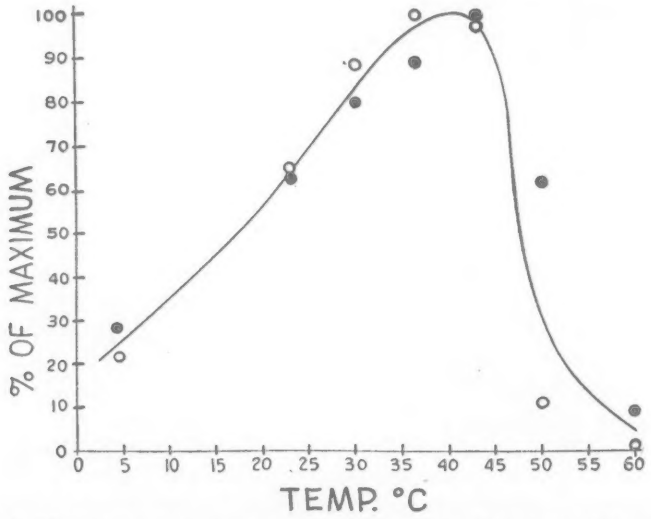

Fig. 3. Effect of temperature on rate of hydrolysis. Time of incubation 6 hours. Solid circles, 6.5 units at maximum. Open circles, 3.2 units at maximum.

taining 5 and 10 units of lipase were 6.8 and 6.6, respectively. Substitution of the M/15 phosphate buffer by M/3 phosphate buffer *p*H 7.0, the strength used by Comfort and Osterberg (5), by 0.4 M NH_4OH—NH_4Cl buffer *p*H 8.4, and by 0.2 M trishydroxymethylaminomethane (THMAM) buffer *p*H 8.4 (23) produced the results shown in Table 3. With the latter two buffers the reaction mixture was adjusted to *p*H 8.4 prior to incubation. The strength of phosphate buffer did not appear to influence results, nor did the use of either of the other two buffers at *p*H 8.4.

Enzyme Concentration

In Fig. 4 the degree of hydrolysis (units) is plotted against the volume of serum used in the test. The concentrations of substrate and buffer were kept constant. The relationship is not linear.

Activators

As determined by studies on pancreatic juice or extract, calcium ions (3) and bile salts (3, 24) activate "lipase." It is believed that the fatty acids released during hydrolysis inhibit enzyme activity, and calcium ions act by removal of the fatty acids through formation of unionized or insoluble soaps. A solution of oxgall in glycerine was prepared by dissolving 5 Gm. oxgall (Bacto) in 5 ml. water, adding 50 ml. glycerine and heating in boiling water. Figure 4 shows the effect of adding 1 ml. of this bile solution to the reaction mixture. It would appear that there was a slight activation. To determine the effect of calcium ions, the technic proposed by Nothman and co-workers (25) was compared with the technic employing M/15 phosphate buffer. The Nothman reaction mixture contains 3.0 ml. water, 5.0 ml. 2% calcium acetate, 5.0 ml. 0.5% sodium barbital, and 1.0 ml. serum. Results obtained with this mixture (Table 4)

Table 3. The Effect of Different Buffers on Results Obtained by the Olive-Oil Method

Sample	*Buffer and initial pH*			
	Phosphate pH 7.0		*NH_4OH—NH_4Cl pH8.4*	*THMAM pH8.4*
	M/15	*M/3*		
1	4.3	..	4.7	..
2	3.4	3.3	..	..
3	1.4	1.4	..	..
4	1.3	1.3	1.3	..
5	2.2	1.9	..	..
6	3.3	3.2	2.6	2.9
7	2.0	1.8	1.8	2.2

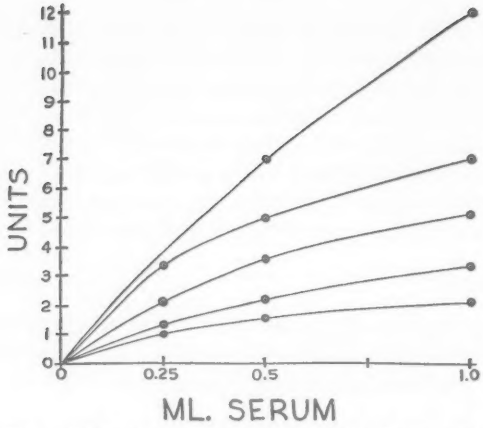

Fig. 4. Effect of enzyme concentration (5 different sera).

Table 4. The Effect of Bile and Calcium on Results Obtained by the Olive-Oil Method

Sample	*M/15 phosphate*	*M/15 phosphate + 1 ml. bile*	*Nothman with calcium acetate*	*Nothman without calcium acetate*
1	10.8	13.9	6.1	12.0
2	4.6	4.9	0.9	5.0
3	3.8	4.5	1.3	4.3
4	1.4	1.3	..	..

were considerably lower than those obtained with phosphate buffer. When the calcium acetate was omitted and water substituted the results agreed quite well with those obtained with phosphate. The addition of 2.5 ml. 2% calcium chloride to a reaction mixture in which the phosphate buffer was replaced by THMAM buffer, *p*H 8.4, also resulted in considerable inhibition. It is concluded, therefore, that calcium ions, at least in the concentrations employed, inhibit rather than activate the lipase present in sera from patients with acute pancreatitis.

Effect of Hemolysis

It has been reported (20) that hemoglobin is an inhibitor of "pancreatitis lipase." This was confirmed in a series of experiments in which it was found that although a serum hemoglobin concentration of 0.16% produced no inhibition, at about 0.5% there was about 50 per cent inhibition and at 2.5% there was almost complete inhibition.

Reproducibility

As determined from 8 replicates on each of two sera containing 3.5 and 5.5 units of activity, the precision (95 per cent limits) was ±10 per cent.

Stability of Samples

Six sera, ranging in activity from 4 to 10 units, were followed at room temperature for one week and no change in activity was observed. One of these was followed for an additional week during which time it showed heavy bacterial growth and an increased lipolytic activity, presumably due to bacterial action. Sera stored at refrigerator temperature were followed for 3 weeks and showed no change in activity.

Normal Values

The 95 per cent limits of the normal range as determined on 24 adults by the "normal equivalent deviate" method (26) after log transformation of the data, were as follows: 0.06 to 0.89 unit for a 4-hour incubation period and 0.2 to 1.5 units for a 16 to 24-hour incubation period. Since the lower limits differ from 0 units by less than the experimental error of the method, the normal ranges can be taken as "up to 0.9 unit" and "up to 1.5 units," respectively.

Bunch and Emerson (9), using essentially the same technic as employed here for the 4-hour technic, obtained limits of 0.06 to 0.87 units. Their limits for a 24-hour technic were 0.1 to 1.0, but they used the calcium-barbital buffer system of Nothman (25) for this series. Comfort and Osterberg (7) considered the top normal to be 1.3 units for the Cherry-Crandall technic.

DISCUSSION

The data presented clearly reveal the striking specificity of the lipolytic enzyme or enzymes occurring in sera from patients with acute pancreatitis. Since pancreatitis lipase differs considerably from lipase in pancreatic extracts with reference to substrate specificity and activators, it is evident that caution should be taken in applying data obtained from work on pancreatic lipase to the problem of the determination of lipase in pancreatitis serum. To avoid confusion in terminology, the term "pancreatitis lipase" is suggested, recognizing the possibility that the pancreas may not be the sole source of the enzyme since elevated values by the olive-oil method have been reported in liver disease (6, 7).

Whether or not the enzyme present in normal serum is the same as that

appearing in pancreatitis serum is not certain. There seems to be no question but that normal sera do have weak activity in hydrolyzing olive oil. Others (27) have agreed with this conclusion.

This study has ended up more or less where it started, namely, with the Cherry-Crandall technic, using an olive-oil emulsion as substrate. The only modification made has been the titration of the liberated fatty acids to *p*H 10.5 either by use of a *p*H meter or by use of thymolphthalein as indicator.

Seligman and Nachlas (28) have proposed a method employing β-naphthyl laurate as substrate, and they reported obtaining increased values in acute pancreatitis. Since this has been questioned (29), and relatively little data are as yet available (30), this method must as yet be considered to be in the investigative stage.

SUMMARY

The specificity of the lipolytic activity occurring in sera from patients with acute pancreatitis for olive oil has been demonstrated. The "lipase" method proposed by Goldstein and co-workers, employing tributyrin as substrate, does not measure "pancreatitis lipase." "Pancreatitis lipase" differs from the enzymes present in pancreatic extracts so that, if the former is the subject of concern in experimental studies, sera from patients with pancreatitis must be used as the source of the enzyme.

Several variables involved in the use of olive oil as substrate have been examined, and the technic finally arrived at is essentially that proposed by Cherry and Crandall with the exception of the use of thymolphthalein as indicator in the titration. Normal values for this technic were determined.

REFERENCES

1. Rona, P., and Michaelis, L., *Biochem. Z.* **31**, 345 (1911).
2. Willstätter, R. and Memmen, F., *Z. f. physiol. Chem.* **129**, 1 (1923).
3. Willstätter, R., Waldschmidt-Leitz, E., and Memmen, F., *Z. f. physiol. Chem.* **125**, 93 (1923).
4. Cherry, I. S., and Crandall, L. A., Jr., *Am. J. Physiol.* **100**, 266 (1932).
5. Comfort, M. W., and Osterberg, A. E., *J. Lab. Clin. Med.* **20**, 271 (1934).
6. Comfort, M. W., *Am. J. Digest. Dis. & Nutrition* **3**, 817 (1936).
7. Comfort, M. W., and Osterberg, A. E., *Proc. Staff Meet., Mayo Clinic* **15**, 427 (1940).
8. Johnson, T. A., and Bockus, H. L., *Arch. Int. Med.* **66**, 62 (1940).
9. Bunch, L. D., and Emerson, R. L., *Clin. Chem.* **2**, 75 (1956).
10. Ingelfinger, F. J., *New Eng. J. Med.* **235**, 653 (1946).
11. Ritcher, D., and Croft, P. G., *Biochem. J.* **36**, 746 (1942).
12. Mendel, B., and Rudney, H., *Biochem. J.* **37**, 59 (1943).
13. Goldstein, N. P., and Roe, J. H., *J. Lab. Clin. Med.* **28**, 1334 (1943).

14. Lagerlöf, H., *Acta Med. Scand.* **120**, 407 (1945).
15. Lagerlöf, H., *Acta Med. Scand., Suppl.* **196**, 399 (1947).
16. Goldstein, N. P., Epstein, J. H., and Roe, J. H., *J. Lab. Clin. Med.* **33**, 1047 (1948).
17. Goldstein, N. P., and Roe, J. H., *J. Lab. Clin. Med.* **28**, 1368 (1943).
18. Alper, C., in *Standard Methods of Clinical Chemistry*, New York, Academic Press, 1953, Vol. 1, p. 71.
19. Todd, J. C., Sandford, A. H., and Wells, B. B., *Clinical Diagnosis by Laboratory Methods* (ed. 12), Philadelphia, Saunders, 1953, p. 456.
20. Tauber, H., *Proc. Soc. Exptl. Biol. Med.* **90**, 375 (1955).
21. Maclay, E., *Am. J. Med. Tech.* **14**, 197 (1948).
22. Fiore, J. V., and Nord, F. F., *Arch. Biochem.* **23**, 473 (1949).
23. Gomori, G., *Proc. Soc. Exptl. Biol. Med.* **62**, 33 (1946).
24. Loevenhart, A. S., and Souder, C. G., *J. Biol. Chem.* **2**, 415 (1906–7).
25. Nothman, M. M., Pratt, T. D., and Benotti, J., *J. Lab. Clin. Med.* **33**, 833 (1948).
26. Moore, F. J., Cramer, F. B., and Knowles, R. G., *Statistics for Medical Students*, New York, Blakiston, 1951, p. 21.
27. Adams, D. H., and Whittaker, V. P., *Biochem. J.* **44**, 62 (1949).
28. Seligman, A. M., and Nachlas, M. M., *J. Clin. Invest.* **29**, 31 (1950).
29. Delcourt, A. A., Rubin, C. E., Palmer, W. L., and Kirsner, J. B., *J. Lab. Clin. Med.* **42**, 310 (1953).
30. Seligman, A. M., Glotzer, P., and Persky, L., *Surgery* **30**, 923 (1951).

Effect of Various Protein Precipitants on Recoveries of Creatinine Added to Plasma

John F. Van Pilsum and M. Bovis

INCOMPLETE RECOVERIES OF CREATININE added to plasma or serum have been reported by investigators who have used a tungstic acid protein precipitation and the picric acid method of analysis (1–7). Controversy exists as to whether the creatinine is lost by adsorption on the protein precipitate or whether the alkaline picrate formed in these circumstances is modified so that less color is formed. Owen *et al.* have reported that both the true and apparent creatinine values were influenced by the reagent used for protein precipitation (1). The true and apparent creatinine values were calculated after using the picric acid method in conjunction with either Lloyd's reagent, (a hydrated aluminum silicate), or by means of specific creatinine destroying bacteria.

In this investigation, recoveries of creatinine added to whole blood and plasma were determined with various types of protein-free filtrates and by the *o*-nitrobenzaldehyde method of analysis. It was found that complete recoveries of creatine and creatinine added to plasma or whole blood were obtained with the tungstic acid method of protein precipitation. The normal values for creatine and creatinine in whole blood and plasma by the *o*-nitrobenzaldehyde procedure were compared with those obtained by the picric acid method.

METHODS

The *o*-nitrobenzaldehyde procedure for creatine and creatinine was used according to a previous publication (8). The picric acid procedure was a modification of the Folin-Wu method (9). To 2 ml. of the 1:7

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protein-free filtrate was added 1 ml. of freshly prepared alkaline picrate solution (5 volumes of a saturated water solution of picric acid mixed with 1 volume of 10% NaOH). The tubes were allowed to stand 15 minutes at room temperature and the color intensities read at 520 mμ. Creatine was measured after conversion to creatinine. To 1 ml. of 1:7 tungstic acid filtrate was added 0.2 ml. 1.0N HCl and the mixture autoclaved for 20 minutes at 120°. After being cooled to room temperature, 1 ml. of alkaline picrate was added, the tubes were allowed to stand 8–10 minutes, 1 ml. of water was added to each, and the color intensities were measured.

A Bausch and Lomb "Spectronic 20" spectrophotometer with ½-in. cuvets was used for all measurements.

RESULTS AND DISCUSSION

Various modifications of the tungstic acid protein precipitation procedure were performed and the recoveries are shown in Table 1. The

Table 1. Recoveries of Creatinine Added to Normal Human Oxalated Plasma with Various Types of Tungstic Acid Protein Precipitation

Volume of				*Method of creatinine Detn.*	*Per cent recovery*	*References*
Plasma	*Water*	*10% Na tungstate*	*⅔ N H_2SO_4*			
1.0	1.5	0.5	1.0	o-nitro	98	
				picric acid	100, 100, 99, 100, 100	1, 2, 4, 5, 6
				picric + LR	100, 98	1, 5
				picric + NCB	100	1
1.0	2.0	1.0	1.0	o-nitro	97	
				picric acid	78, 88	4, 5
				picric + LR	81	5
1.0	7.0	1.0	1.0	o-nitro	102	
				picric acid	88, 85, 90	3, 4, 5
				picric + LR	91	5
1.0	2.0	0.5	0.5	o-nitro	99	
				picric acid	90, 78, 86	2, 3, 6
1.0	3.0	0.5	0.5	o-nitro	98	
				picric	89, 97	1, 5
				picric + LR	94, 94	1, 5
				picric + NCB	91	1
1.0	8.0	0.5	0.5	o-nitro	101	
				picric	94	4
1.0	1.0	1.0	1.0	o-nitro	97	
1.0	12.0	1.0	1.0	o-nitro	100	
1.0	17.0	1.0	1.0	o-nitro	98	
1.0	4.0	1.0	1.0	o-nitro	100	

Recoveries with the o-nitrobenzaldehyde procedure were determined with an added 5.0 mg./100 ml. creatinine.

LR = Lloyd's Reagent.

NCB = Creatinine Destroying Bacteria.

average recovery of creatinine added to plasma was 99 per cent. The results of this investigation indicate that creatinine is not lost by adsorption on the protein precipitate as has been suggested (1, 3, 7). The reasons for the incomplete recoveries by other investigators with the picric acid method of analysis are not known.

Table 2. Recoveries of Creatine and Creatinine Added to Normal Human Oxalated Whole Blood and Plasma with a Tungstic Acid Precipitation and the o-Nitrobenzaldehyde Method of Analysis

mg./100 ml. added	*Whole blood*		*Plasma*	
	Detn. mg./100 ml.	*% Recovery*	*Detn. mg./100 ml.*	*% Recovery*
Creatine				
0	3.1		0.3	
1	4.2	103	1.3	100
3	6.0	98	3.4	103
5	8.1	100	5.4	102
10	12.9	98	10.3	100
Creatinine				
0	0.8		0.8	
1	1.8	100	1.8	100
3	3.8	100	3.7	97
5	5.8	100	5.8	100
10	10.7	99	10.5	97

To 1 volume of oxalated whole blood was added 3 volumes of distilled water or creatine or creatinine standard solutions. 1½ volumes of 10% Na_2WO_4 was added followed by 1½ volumes of ⅔ N H_2SO_4. After standing 10 minutes, the mixture was centrifuged and filtered. When plasma was used, 4 volumes of water or creatine or creatinine standard solutions was added followed by 1 volume each of 10% Na_2WO_4 and ⅔ N H_2SO_4.

Table 3. Recoveries of Creatinine Added to Normal Human Oxalated Whole Blood and Plasma with Various Types of Protein Precipitation and the o-Nitrobenzaldehyde Method of Analysis

Type of protein precipitation	*Whole blood*		*Plasma*	
	mg./100 ml.	*% Recovery*	*mg./100 ml.*	*% Recovery*
1. 1½ vol. 10% Na_2WO_4, 1½ vol. ⅔ N H_2SO_4[a]	6.1	100	6.1	100
2. 3 vol. 0.3 N $Ba(OH)_2$, 3 vol. 5% $ZnSO_4$	2.0	33	2.0	33
3. 3 vol. 0.3 N NaOH, 3 vol. 5% $ZnSO_4$	3.6	59	4.0	65
4. 3 vol. 0.3 N NaOH, 3 vol. 2.2% $CdSO_4$	6.1	100	6.0	98
5. 2 vol. 20% trichloroacetic acid	6.1	100	6.0	98
6. 4 vol. 0.6 N perchloric acid	6.2	102	6.0	98

5 mg./100 ml. of creatinine added to the blood or plasma. To 1 volume of whole blood or plasma was added the above volumes of precipitating agents. A 1:7.5 protein-free filtrate made. Protein-free filtrates that were not at pH 3–4 were so adjusted by the addition of acid or alkali before the analyses were made.

[a] With plasma, 1 volume each of 10% Na_2WO_4 and ⅔ N H_2SO_4 was used.

Table 4. CREATINE AND CREATININE IN NORMAL ADULT MALE WHOLE BLOOD AND PLASMA

	No. samples	*o-Nitrobenzaldehyde method (mg./100 ml.)*	*Picric acid method (mg./100 ml.)*
Whole-blood creatine	14	2.7 ± 0.25[a]	2.8 ± 0.44
Whole-blood creatinine	20	0.6 ± 0.12	1.4 ± 0.13
Plasma creatine	16	0.2 ± 0.15	0.5 ± 0.21
Plasma creatinine	24	0.8 ± 0.11	1.0 ± 0.14

All determinations made with a 1:7 tungstic acid protein-free filtrates.

[a] Standard deviations $\sigma = \sqrt{\frac{\Sigma(X^2)}{N}}$

where X = deviations from arithmetic mean.
N = total number of items.

In Table 2 are shown the recoveries of creatine and creatinine added to whole blood and plasma with a tungstic acid protein precipitation. The average recovery of the added compounds was 99 per cent.

In Table 3 are shown the recoveries of creatinine added to whole blood and plasma when other types of protein precipitation were used. Complete recoveries were obtained with $CdSO_4$ + NaOH, trichloroacetic acid, and perchloric acid. Incomplete recoveries were obtained with $Ba(OH)_2$ + $ZnSO_4$, and NaOH + $ZnSO_4$. Hare also has reported complete recoveries with tungstic acid, $CdSO_4$, and trichloroacetic acid (10).

Table 4 shows the values for creatine and creatinine in whole blood and plasma by the picric acid and by the *o*-nitrobenzaldehyde procedures. There was no significant difference in the whole-blood creatine values by either method. The difference between the whole-blood creatinine, plasma creatine, and the plasma creatinine values by the two methods was statistically significant. The values were 43, 40, and 80 per cent of the picric acid values, respectively. Previous investigators have calculated the true serum creatinine content to be about 80–100 per cent and the true erythrocyte creatinine content to be about 30–50 per cent of the values obtained by the picric acid method. The true serum creatine was calculated to be about 70 per cent and the true erythrocyte creatine to be about 85–90 per cent of the picric acid values (1, 11, 12).

Thus, this work is a confirmation of the results obtained by other investigators who have used Lloyd's reagent or creatinine-destroying bacteria to show that the plasma and whole-blood creatinine values obtained by the picric acid method are exaggerated by the presence of noncreatinine, picric acid-positive chromogens.

SUMMARY

The effect of various types of protein precipitation procedures upon the recovery of creatinine added to blood has been investigated. Complete

recoveries of creatinine were obtained with tungstic acid, trichloroacetic acid, perchloric acid, and cadmium hydroxide types of protein precipitation. Incomplete recoveries were obtained with $Ba(OH)_2 + ZnSO_4$ and $NaOH + ZnSO_4$ types of protein precipitation.

A comparison was made of the normal values for creatine and creatinine in whole blood and plasma obtained by the picric acid and by the *o*-nitrobenzaldehyde methods of analysis. The *o*-nitrobenzaldehyde values were 97, 43, 40, and 80 per cent of the picric acid values for whole-blood creatine, whole-blood creatinine, plasma creatine, and plasma creatinine, respectively.

REFERENCES

1. Owen, J. A., Iggo, B., Scandrett, F. J., and Stewart, C. P., *Biochem. J.* **58**, 426 (1956).
2. Brod, J., and Sirota, J. H., *J. Clin. Invest.* **27**, 645 (1948).
3. Camara, A. A., Arn, K. D., Reimer, A., and Newburgh, L. H., *J. Lab. Clin. Med.* **37**, 743 (1951).
4. Haugen, H. N., and Blegen, E. M., *Scand. J. Clin. Lab. Invest.* **5**, 58 (1953).
5. Mandel, E. E., and Jones, F. L., *J. Lab. Clin. Med.* **41**, 323 (1953).
6. Roscoe, M. H., *J. Clin. Path.* **6**, 201 (1953).
7. Lauson, H. D., *J. Applied Physiol.* **4**, 227 (1951).
8. Van Pilsum, J. F., Martin, R. P., Keto, E., and Hess, J., *J. Biol. Chem.* **222**, 225 (1956).
9. Folin, O. and Wu, H., *J. Biol. Chem.* **38**, 81 (1919).
10. Hare, R. S., *Proc. Soc. Exptl. Biol. Med.* **74**, 148 (1950).
11. Miller, B. F., and Dubos, R., *J. Biol. Chem.* **121**, 457 (1937).
12. Allison, M. J. C., *J. Biol. Chem.* **157**, 169 (1945).

Comparison of the Archibald–Kern and Stransky Colorimetric Procedure and the Praetorius Enzymatic Procedure for the Determination of Uric Acid

Carl Alper and Joseph Seitchik

THE ESTABLISHMENT AND ACCEPTANCE of an analytic procedure as a method to be relied upon with complete confidence demands: (1) *specificity of the method*, i.e. minimal interference by other compounds; (2) *quantitative recovery* of the specific substance when it is added to the medium in which it is usually determined; and (3) *reproducibility of results* within reasonable limits on replicate samples.

The colorimetric methods for the determination of uric acid depend upon the mild oxidation of uric acid in an alkaline or acid medium by molybdate-free phosphotungstic acid (1, 2), arsenotungstic acid (3, 4), or ferricyanide (5, 6, 7). The latter method has been used in combination with the specific enzyme uricase. Among the sources of error associated with these procedures are the inhibition of the uric acid reaction by some of the blood constituents and the precipitation of uric acid with the serum protein in the preparation of a protein-free filtrate, which would yield results below those obtained by uricase. The presence of other substances in blood filtrates which may also reduce the nonspecific oxidizing agents

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and the occurrence of turbidity due to instability of reagents would yield results greater than those obtained by uricase. Lous and Sylvest (8) have demonstrated that most of the results of 188 samples of serum determined by the Folin procedure and compared with results of the enzymatic procedure are less than those obtained by uricase. On the other hand, of 233 samples of serum assayed by the titrimetric method and compared with the enzymatic method, all of the results are greater than those obtained by the uricase method. Indeed, it is interesting to note that, as chemical technics are perfected, the average figures of blood uric acid levels have become greater, whereas the normal range of values has become more limited.

Since there are interfering substances such as ascorbic acid, glucose, amino acids, and glutathione normally present in the blood and urine which can be readily oxidized, the analytic problem is twofold: (1) the selection of an oxidizing agent in the proper medium with an oxidation-reduction potential which will just satisfy the demands for the oxidation of uric acid and (2) the selection of a system for the development of color which will be specific stoichiometrically for the products of oxidation of uric acid, the unused oxidizing agent, or the reduced form of the oxidizing agent. When this has been accomplished, one can then evaluate the over-all qualitative and quantitative specificity of the method for uric acid.

It would be desirable also to compare this method with a procedure of known specificity, one which may not lend itself to the routine clinical chemistry laboratory. The problem is resolved then in the use of the Archibald modification (10) of the Kern and Stransky (11) method for the assay of uric acid in blood and a comparison of results obtained by this method with those obtained by the enzymatic method of Praetorius (12, 13).

METHODS

A detailed account of the colorimetric procedure for the determination of uric acid is described by Archibald (10) in an accompanying paper in this issue of CLINICAL CHEMISTRY. The enzymatic procedure was described by Praetorius (12, 13).

RESULTS

Spectral Characteristics

In photometric analysis the percentage of light transmitted through a colored solution is related to the concentration of the light-absorbing

material. Figure 1 demonstrates that the blue color which develops from the reduction of phosphotungstic acid by uric acid follows Beer's law. The color formation is a function of time and the readings on the photometer should be taken at exactly 15-minute intervals after the addition of the special uric acid reagent. The absorbance measurements are obtained on a spectrophotometer with light of wave length of 700 mμ, or with the aid of a 660 red filter in a photoelectric colorimeter.

Reliability of the Method

The specificity of the colorimetric method is demonstrated by the quantitative recovery of uric acid added to serum. The results depicted in Table 1 illustrate that the excellent recovery of uric acid by this procedure is of the same order of accuracy as that observed for the enzymatic procedure.

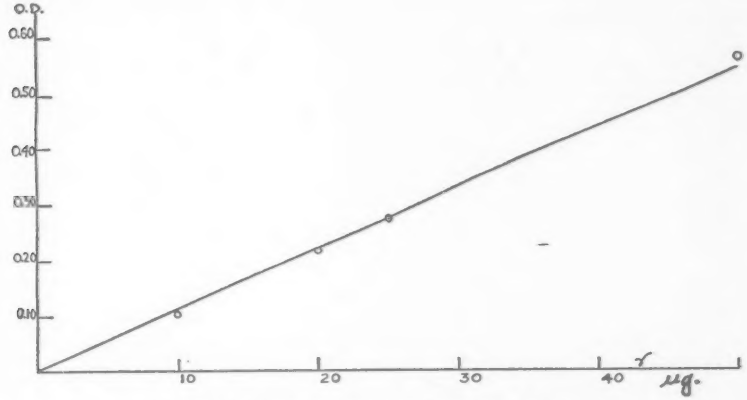

Fig. 1. Standard curve of serum uric acid.

Table 1. DETERMINATION OF URIC ACID
Recovery from Serum

Serum Conc. + (μg./ml.)	*Archibald dein.* (μg./ml.)	*Per cent recovery*	*Serum Conc.* + (μg./ml.)	*Praetorius dein.* (μg./ml.)	*Per cent recovery*
0	47.2	..	0	52.6	..
5	52.2	100	8.1	60.2	99.1
10	57.2	100	13.25	65.8	100
15	61.6	99.3	27.3	81.0	101
20	68.0	101	37.7	90.0	99.6
25	71.9	99.5			

A comparison of results obtained by both procedures on the same sample of serum in sixty-one instances demonstrates that the Archibald colorimetric procedure yields results which are 96.1 ± 1.4 per cent of the result obtained by the specific enzymatic procedure. The actual range is 94.3 per cent to 98.0 per cent of the enzymatic procedure, which is less than two standard deviations of the mean percentage.

Duplicate analysis of serum uric acid is demonstrated most satisfactorily by a scattergram (Fig. 2), which illustrates linear correlation in 109 duplicate determinations. The determination of the standard deviation for duplicate analyses yields a value of 0.1 according to the formula of Youden (14).

$$\text{S.D.} = \frac{1}{2n} \times d^2$$

where d is the differences between duplicates and n is the number of degrees of freedom.

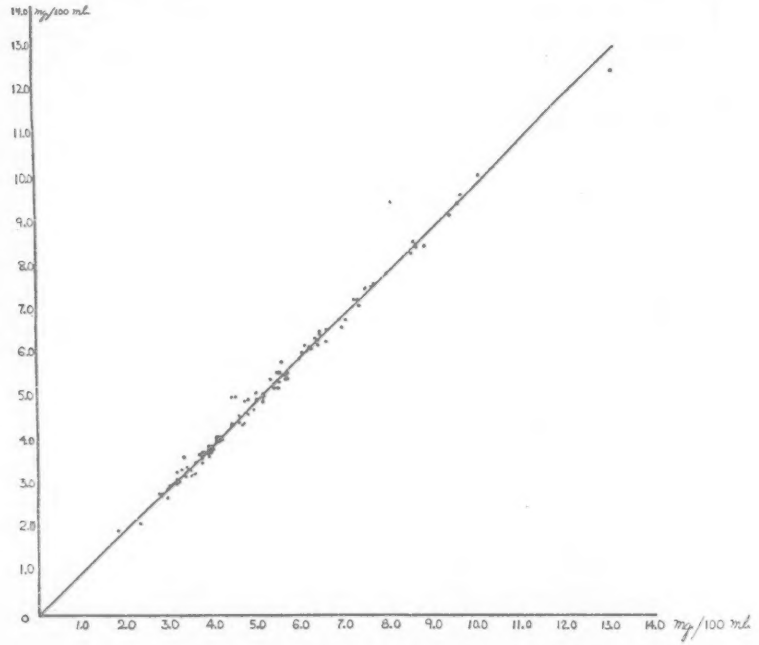

Fig. 2. Duplicate analysis of serum uric acid.

$$\text{S.D.} = \frac{2.3984}{2 \times 109} = 0.1$$

Normal Distribution of Values

A study of the distribution of values on 143 samples of serum from blood donors to the American Red Cross demonstrates a bimodal distribution (Fig. 3). This would indicate the presence of two distinct population groups based on a parameter which is sex-linked. The mean value for a male group of 95 samples is 5.4 ± 0.82 mg./100 ml. of serum; whereas the mean value for a female group of 48 samples is 4.0 ± 0.72 mg./100 ml. serum. A determination of the significance of the difference between the two means (15) presented in Table 2 yields a value for t which demonstrates that the mean values represent results derived from two distinct population groups.

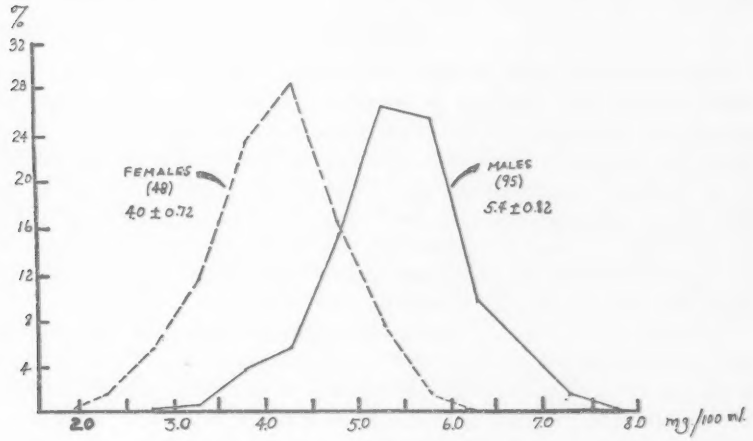

Fig. 3. Distribution of values of serum uric acid.

Table 2. Determination of Uric Acid
Significance of the Difference Between Two Means

	Males	*Females*
No. of observations	95	48
Mean	5.4 μg/ml. ±0.82	4.0 μg/ml. ±0.72
—	$t = \frac{\bar{X}_{\text{males}} - \bar{X}_{\text{females}}}{\text{S. E. of difference}}$	
	$t = 9.9$	

Table 3. Uric Acid Level in Serum (Apparently Normal Individuals)

Author	*Method and Reference*	*Sex of Subject*	*No. observed*	*Mean value (μg./ml.)*	*Range (μg./ml.)*
Brøchner-Mortensen (7)	(7)	M	33	7.6	..
		F	37	6.4	..
Natelson and Kaser (18)	Brown (2)	M + F	51	4.3	2.4–5.9
Gjørup, Poulsen, and Praetorius (16)	Praetorius (13)	M	157	5.04	2.6–7.5
		F	143	3.84	2.0–5.7
Alper and Seitchik	Archibald (10)	M	95	5.4	3.8–7.1
		F	48	4.0	2.6–5.4

Although the sex difference in plasma or serum uric acid concentration has been noted previously (7, 16, 17), in general it has been overlooked, as evidenced by the values reported in Natelson and Kaser (18). The values reported in Table 3 agree well with results based upon the enzymatic procedure.

DISCUSSION

From a clinical point of view, the determination of serum uric acid with precision and accuracy is desirable. There is no question that an enzymatic procedure would fulfill these requirements. On the other hand, the enzymatic procedure, either as devised by Praetorius (12, 13) or as modified by Feichtmeir and Wrenn (17), requires the use of an ultraviolet spectrophotometer. Such equipment is not always available in the clinical chemistry laboratory. Consequently, it is desirable to provide a colorimetric procedure which approximates or very closely approximates the specific enzymatic procedure.

The colorimetric method devised by Archibald (10) has fulfilled all of the requirements set forth for the establishment of the reliability of an analytical procedure. It is specific in respect to recovery, lack of interference by other reducing substances found in serum in usual quantities, and in comparison with the specific enzymatic procedure. In addition the method is rapid and easily carried out.

The objections raised by Feichtmeir and Wrenn (17) to the Archibald modification of the Kern and Stransky procedure can be overcome if the procedure as described is followed exactly. Feichtmeir (18) in adapting the Archibald procedure did not alkalinize the plasma prior to precipitation of protein, prepared a more acid serum filtrate, and used a different proportion of reagents to develop color. Alkalinization of the serum may be expected to destroy interfering substances such as ascorbic acid or sulfhydryl groups. The acidity of the serum or plasma filtrate and the proportions of reagents will affect color formation.

These results are significant in that they provide proof of the adequacy of a procedure which can find universal use in clinical chemistry.

Great emphasis should be placed on the difference between values for males and females. It is interesting to note that the blood levels of uric acid in pregnancy fall within the range for males. At this point we offer no explanation for this fact. It is rather difficult to assign limits for normal range of values. We agree with Sjørup, Poulsen, and Praetorius (16) that this is an arbitrarily defined limitation and further concur that two times the standard deviation of a single observation which should include 95.5 per cent of all observations would make desirable limits.

SUMMARY

The Archibald modification of the Kern and Stransky procedure for uric acid has been studied and its efficacy for routine use in clinical chemistry evaluated. Mean values and the range of values for men and women have been determined.

REFERENCES

1. Folin, O., *J. Biol. Chem.* **101**, 111 (1933); **106**, 311 (1934).
2. Brown, H., *J. Biol. Chem.* **158**, 601 (1945).
3. Benedict, S. R., *J. Biol. Chem.* **51**, 189 (1922); **54**, 233 (1922).
4. Newton, E., *J. Biol. Chem.* **120**, 315 (1937).
5. Blauch, M. B., and Koch, F. C., *J. Biol. Chem.* **130**, 443 (1939).
6. Bulger, H. A., and Johns, H. E., *J. Biol. Chem.* **140**, 427 (1941).
7. Brøchner-Mortensen, K., *Medicine* **17**, 161 (1940).
8. Lous, P., and Sylvest, O., *Scand. J. Clin. and Lab. Investigation* **6**, 40 (1954).
9. Forsham, P. H., Hills, A. G., Thorn, G. W., and Prunty, F. T. G., *J. Clin. Endocrinology* **8**, 15 (1948).
10. Archibald, R. M., *Clin. Chem.* **3**, 102 (1957).
11. Kern, A., and Stransky, E., *Biochem. Z.* **290**, 419 (1937).
12. Praetorius, E., *Scand. J. Clin. and Lab. Investigation* **1**, 222 (1949).
13. Praetorius, E., and Poulsen, H., *Scand. J. Clin. and Lab. Investigation* **5**, 273 (1953).
14. Youden, W. J., *Statistical Methods for Chemists*, New York, Wiley, 1951, pp. 13–17.
15. Chambers, E. G., *Statistical Calculations for Beginners*, Cambridge, England, 1940, pp. 29–39.
16. Gjørup, S., Poulsen, H., and Praetorius, E. *Scand. J. Clin. and Lab. Investigation* **7**, 201 (1955).
17. Feichtmeir, T. V., and Wrenn, H. T., *Am. J. Clin. Path.* **25**, 833 (1955).
18. Natelson, S., and Kaser, M., in M. Reiner: *Standard Methods of Clinical Chemistry*, New York, Acad. Press, 1953, pp. 123–135.
19. Feichtmeir, T. V. Personal communication.

Colorimetric Measurement of Uric Acid

R. M. Archibald

WHEN ASKED IN 1942 to find a method for the determination of plasma or serum uric acid suitable for inclusion in the War Department's Technical Manual 8-227, *Methods for Laboratory Technicians*, the author believed that the procedure of Kern and Stransky (1) filled the requirements admirably. In this modification, toxic and heat-labile sodium cyanide solution was replaced by the effective but nontoxic and relatively stable glycerine-silicate reagent. However, when the method of Kern and Stransky was used exactly as directed, colored solutions quickly became turbid when the glycerine-silicate used was prepared from either a diluted water-glass preparation or from a solid silicate. Furthermore, use of any one of the available preparations of glycerine resulted in a reagent mixture which permitted development of turbidity. As Kern and Stransky made no mention of such turbidity, it seems likely that the glycerine preparations available to them contained some impurity which inhibited precipitation from the glycerine-silicate mixture. In any case, addition of Liquoid La Roche, a Swiss preparation of sodium polyanethol sulfonate, consistently prevented development of turbidity in the colored solutions. No adequate substitute for this sulfonate was found. The first few batches of Liquoid prepared in this country were also unable to prevent development of turbidity. However, batches of Liquoid marketed by Hoffman-La Roche, Inc., during the past ten years appear to be completely satisfactory.

To decrease the number of reagents required for conduct of this procedure, the protein precipitant (uranyl acetate) employed by Kern and Stransky was replaced by the reagent used for the development of color. Thus, the phosphotungstic acid reagent serves not only for the development of color, but also for the precipitation of blood proteins.

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METHOD

Reagents

Standard solution of uric acid. Approximately 0.5 Gm. lithium carbonate, dissolved in 150 ml. of hot water is added with stirring to exactly 1 Gm. of uric acid. The solution is transferred with 300 ml. of rinsings to a liter volumetric flask; 25 ml. of 40 per cent formaldehyde, then 3 ml. of glacial acetic acid are added with mixing. After the liberated CO_2 has been removed from solution by shaking, water sufficient to bring the volume to 1 liter is added and mixed. This stock standard, if protected from light, is stable for at least 1 year. One milliliter of this stock, diluted to 200 ml. with water on the day of use, serves as a working standard, and contains 0.005 mg. uric acid per ml.

Polyanethol sodium sulfonate. 1 Gm. of Liquoid La Roche is dissolved in 50 ml. of water. The solution is stored in a refrigerator.

Glycerine-silicate reagent. 10 Gm. of Merck's crystalline sodium silicate "soluble" is dissolved in 100 ml. of hot water, then mixed with 20 ml. of glycerine. Alternatively, 130 ml. of Merck's water-glass dissolved in 250 ml. of water, and 85 ml. of reagent-grade glycerine are mixed at room temperature and made up to 500 ml. with water. If the solution prepared by either procedure is cloudy, it should be filtered through hardened paper and stored in a Pyrex container.

Phosphotungstic acid solution. 50 Gm. of reagent-grade sodium tungstate, (Na_2WO_4—$2H_2O$), 400 ml. of water, and 40 ml. of 85 per cent orthophosphoric acid are boiled under a reflex condenser for 4 hours, then made up to a volume of 500 ml. with water and stored, protected from light. Commercial phosphotungstic acid should not be substituted for this solution.

Sodium hydroxide solution. 0.5N.

Procedure

One volume of plasma or serum, 8 volumes of water, and 0.4 volumes of 0.5N NaOH are mixed by whirling for about 1 minute in a small Erlenmeyer flask. Then 0.6 volumes of phosphotungstic acid solution are added with mixing. The first half may be added rapidly but the last half should be added dropwise with constant stirring or whirling to insure complete precipitation of protein. After 5 minutes the mixture is filtered through a Whatman #42 filter paper or centrifuged. To 10 volumes of the filtrate or supernatant fluid, 5 volumes of glycerine-silicate reagent and 1 volume of polyanethol sodium sulfonate solution are added with

constant mixing.[1] Finally 1 volume of the phosphotungstic acid solution is added with instantaneous mixing. After 15 minutes the color is fully developed and remains almost constant for half an hour. The absorbance measurements are obtained on a spectrophotometer at 700 mμ, or with the aid of a #660 red filter in a photoelectric colorimeter. Ten volumes of diluted standard and 10 volumes of water are each treated in the same manner as the plasma filtrate to provide a standard and photometric blank, respectively.

CALCULATIONS

$$\text{Mg. uric acid per 100 ml. plasma or serum} = 5 \times \frac{\text{absorbance of unknown}}{\text{absorbance of standard}}$$

DISCUSSION

Because use of glycerine-silicate reagent did away with the need for refrigeration of most reagents, and because no special protein precipitating reagent was required, this modification seemed one that might be of particular use in advanced service bases with limited cold-storage conveniences. Use of this procedure permitted excellent recovery of added uric acid, and was attended by insignificant interference from amounts of ascorbic acid, ergothioneine, glutathione, or cystine, likely to be encountered in the blood. Hence, the method appeared to be very adequate for clinical evaluation of the uric acid levels of plasma or sera, and it was submitted for use in the War Department's Technical Manual (2), which had at that time a restricted circulation. It was believed by the author that proof of adequacy of the procedure was not yet sufficient to justify its publication as a general method for investigative work. The wisdom of its use for the measurement of urate in urine was especially in doubt. However, the procedure was acquired by the Coleman Electric Co. and included in the manual for use with the Coleman Junior Clinical Spectrophotometer Army Model (3). Because of the simplicity and convenience of the method, it soon gained favor, even among research workers who have demonstrated the applicability of the method to the measurement of urine urate (4), and have offered convincing proof of its reliability in the measurement of urate in blood (5).

The addition of alkali before precipitation of proteins probably increases the specificity of the method considerably. The high *p*H thus

[1] Forsham *et al.* (4) found it convenient to mix 5 parts of Liquoid, 4 parts of uric acid reagent, and 9 parts of water every two weeks, and to add 4 volumes of this mixture to each of the unknowns, standards, and blanks rather than to add the Liquoid and uric acid reagents separately.

obtained might be expected to permit oxidative destruction of interferring substances such as ascorbate and sulfhydryl compounds. In any case, use of a modification of the method which omits such preliminary treatment with alkali has resulted in reports of some values which appear to deviate appreciably from the true uric acid levels (6).

SUMMARY

A convenient modification of the uric acid method devised by Kern and Stransky has been described. Its reliability has been demonstrated largely by other workers.

REFERENCES

1. Kern, A., and Stransky, E., *Biochem. Z.* **290**, 419 (1937).
2. "Uric Acid in Plasma or Serum," In the U. S. Army Department, *Methods for Medical Laboratory Technicians* (Technical Manual TM 8-227; Air Force Manual AFM 160-14), Washington, D. C., U. S. Govt. Print. Office, 1951, p. 232. Originally described in U. S. Army Technical Manual TM 8-227, 1946.
3. "Estimation of Uric Acid in Plasma or Serum" (TE 38-21), in Coleman Electric Company, *Clinical Methods for the Coleman Junior Clinical Spectrophotometer Model 6, Army Model*, Maywood, Ill., 1944.
4. Forsham, P. H., Thorn, G. W., Prunty, F. T. G., and Hills, A. G., *J. Clin. Endocrinol.* **8**, 15 (1948).
5. Alper, C., and Seitchik, J., *Clin. Chem.* **3**, 95 (1957).
6. Feichtmeir, T. V., and Wrenn, H. T., *J. Clin. Path.* **25**, 833 (1955).

Determination of L-Tyrosine as Tyramine in Urine

Richard E. Tashian

THE METHODS HITHERTO AVAILABLE for the quantitative estimation of *l*-tyrosine in urine have been Millon's reaction, microbiologic technics, paper chromatography, enzyme-manometric assay, and ion-exchange chromatography. The first two methods lack specificity, and the use of paper chromatography, although specific, is not easily quantitated. Lawrie (1) reported a manometric technic based on Epps's (2) discovery of the enzymatic decarboxylation of *l*-tyrosine by *Streptococcus faecalis*. This method, however, is not entirely satisfactory in that *S. faecalis* was later found to act also on *l*-phenylalanine (3). Undoubtedly, the most accurate method is that of ion-exchange chromatography (4), but the length of time required and the relative complexity of the technic are limiting factors. During the course of a study of phenylalanine and tyrosine metabolism in this laboratory a rapid and specific colorimetric assay of *l*-tyrosine was developed based on the nitrosonaphthol method of Udenfriend and Cooper (5).

Because of the presence in human urine of certain interfering coloring substances such as some phenolic acids and reducing agents (e.g., uric acid), it is essential that the tyrosine be separated from the major bulk of urine constituents before employing the nitrosonaphthol assay (5). This can readily be brought about by treating the urine with an acetone-dried preparation of *S. faecalis* which contains tyrosine decarboxylase and converts the tyrosine to tyramine (2). The greater part of the

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tyramine can then be extracted from the incubated mixture and satisfactorily assayed by the nitrosonaphthol technic in which both tyrosine and tyramine react alike. The only other compounds known to be attacked by *S. faecalis* are dihydroxyphenylalanine (DOPA) and phenylalanine, but they give rise to non-nitrosonaphthol products; also DOPA is not present in human urine.

MATERIALS AND REAGENTS

An acetone-dried powder of *S. faecalis* prepared by the methods of Epps (2) and McGilvery and Cohen (3). Store at 0°, or less. The powder is suspended in citrate buffer (see below) prior to use, 20 mg. per ml. of buffer.

0.7M citrate buffer, *p*H 5.5.

NaCl.

1N NaOH.

1N HCl.

Ethyl acetate (reagent grade); other suitable organic solvents may be substituted.

PROCEDURE

To 2 ml. of urine (or 1 ml. diluted to 2 ml. with water) in a 60-ml. glass-stoppered bottle add 0.5 ml. of the *S. faecalis* suspension and 0.5 ml. of 0.7M citrate buffer, *p*H 5.5. Incubate at 37° for 2 hours. After cooling, treat with 0.7 Gm. of NaCl and adjust to *p*H 9–10 by adding 0.5 ml. of 1N NaOH. Extract contents of bottle into 10 ml. of ethyl acetate by shaking for 5 minutes. Shaking should be vigorous in order to prevent gelation. Transfer 8–9 ml. of the ethyl acetate to a 15 ml. glass-stoppered centrifuge tube and extract for 2 minutes into 2 ml. of 1N HCl. Carefully aspirate the ethyl acetate from the top of the aqueous acid layer with a fine-tipped pipette and assay the tyramine in the remaining 2 ml. of HCl by the method of Udenfriend and Cooper (5). Good results were obtained with this final step. However, a modification could be introduced in which the ethyl acetate is extracted into 2.5 ml. of 1N HCl, and a 2-ml. aliquot of the HCl then taken for the tyramine determination.

RESULTS AND DISCUSSION

To test the specificity of this method the absorption spectra of the nitrosonaphthol tyramine derivatives of the unknown and standard were compared in a Coleman Junior spectrophotometer. The curves were found to be identical with peaks occurring at 450 mμ.

Table 1. Recovery of Tyrosine from Urine (1 ml. Used)

Tyrosine added (mg.)	*Tyrosine found* (mg.)	*Per cent recovery*
0.00	.030	
0.01	.039	90
0.01	.041	110
0.02	.049	95
0.02	.049	95
0.03	.059	97
0.03	.061	103

Table 2. Tyrosine Present in 24-Hour Samples of Human Urine

Sex	*Volume* (ml.)	*Tyrosine* (mg.)	*Creatinine* (mg./ml.)	*Tyrosine/ creatinine*
M	850	25.7	1.69	.017
M	1330	26.1	1.13	.017
M	1325	12.1	1.05	.009
F	1275	6.5	0.73	.007

Table 3. Tyrosine Present in Samples of Human Urine

		Tyrosine (mg./100 ml.)		*Tyrosine/creatinine*	
Sex	*No.*	*Range*	*Mean ± standard deviation*	*Range*	*Mean ± standard deviation*
M	17	0.46–8.02	2.81 ± .530	.006–.033	.015 ± .002
F	18	1.07–3.60	2.08 ± .179	.005–.029	.012 ± .001

The urine samples (average 60 ml.) were obtained from normal, healthy individuals ranging in age from 15 to 33 years.

The recoveries of specific amounts of tyrosine added to human urine are shown in Table 1. The average per cent recovery for the 6 determinations was about 98 per cent.

The tyrosine present in four 24-hour samples of human urine are given in Table 2. These values are in general agreement with those found by others. Stein (4) and Evered (6), using ion-exchange chromatography, report values of 15 to 49 mg. in 24 hour urine samples in a combined total of 17 individuals. With the enzyme-manometric and microbiological methods, Lawrie (1) and Woodson *et al.* (7) report levels from 11 to 44 mg.

Recently, investigators in this field have adopted the practice of expressing urinary amino acids in relation to creatinine concentration with the view that this will correct for fluctuations in amino acid concentration due to variation in urine dilution. Accordingly, it can be seen in Table 2 that the tyrosine/creatinine ratios corresponding to the 24-hour samples bear a direct relation to the amount of tyrosine excreted in 24 hours.

This would then seem to support the use of creatinine ratios as a means of representing urinary amino acid concentrations in small urine samples. Table 3 compares the urine tyrosine concentrations in both milligrams per 100 ml. and creatinine ratio in urine samples from 35 individuals.

SUMMARY

A procedure for the determination of *l*-tyrosine in human urine is described. Tyrosine is converted to tyramine by treating it with the decarboxylase present in an acetone-powder preparation of *S. faecalis*. The tyramine is then separated from the urine and assayed by the nitrosonaphthol method. Examples of the application of this technic to human urine are presented.

REFERENCES

1. Lawrie, N. R., *Biochem. J.* **41**, 41 (1947).
2. Epps, H. M. R., *Biochem. J.* **38**, 242 (1944).
3. McGilvery, R. W. and Cohen, P. P., *J. Biol. Chem.* **174**, 813 (1948).
4. Stein, W. H., *J. Biol. Chem.* **201**, 45 (1953).
5 Udenfriend, S., and Cooper, J. R., *J. Biol. Chem.* **196**, 227 (1952).
6. Evered, D. F., *Biochem. J.* **62**, 416 (1956).
7. Woodson, H. W., Hier, S. W., Soloman, J. D., and Bergeim, O. *J. Biol. Chem.* **172**, 613 (1948).

Alpha- and Beta-Lipoprotein Cholesterol

Method of Rapid Quantitative Determination

F. S. Nury and Elizabeth R. B. Smith

A RECENT COMMUNICATION from this laboratory (1) reported a method for the estimation of cholesterol in electrophoretically separated α- and β-lipoproteins. This method required very small amounts of blood serum but necessitated special equipment and operations extending over more than one day. Improvements have now been made in the procedure which eliminate specially built apparatus and shorten the time required for analysis so that "same-day" reports can be made in a clinical laboratory.

REAGENTS

Barbital Buffer pH 8.6, 0.005 μ: 1.84 Gm. diethylbarbituric acid and 10.3 Gm. sodium diethylbarbiturate dissolved in 1 L. distilled water.

Staining Solution 1 Gm. Sudan Black B (National Aniline Division, Allied Chemical and Dye Corporation) (2) in 1 L. 60% alcohol. The mixture is brought to the boiling point, cooled to room temperature, filtered, and stored in a tightly closed container.

Elution Fluid Two volumes of redistilled chloroform and one volume of redistilled methanol mixed just prior to use.

Glacial Acetic Acid Fisher reagent grade, checked for the absence of glyoxylic acid by the method of Brice (3).

Ferric Chloride–Sulfuric Acid Reagent 1 Gm. reagent grade $FeCl_3 \cdot 6H_2O$ dissolved in 10 ml. glacial acetic acid. Five milliliters of this solution is diluted to 500 ml. with reagent grade H_2SO_4.

From the Department of Pharmacology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.

The authors are indebted to Dr. W. P. Jencks for suggestions and to Mr. Rudolph Frei for the Sperry and Webb cholesterol measurements.

Received for publication February 4, 1956.

Standard Cholesterol Solution 100 mg. recrystallized cholesterol dissolved in glacial acetic acid and made up to 100 ml. at 25°. This standard was kept frozen when not in use.

PROCEDURE

Electrophoresis

Separations were carried out on 2.9- by 30.0-cm. strips of Whatman 3 MM filter paper in a cell of the hanging-strip type (4). The strips were saturated with buffer and allowed to drain 10 minutes; a micropipet was then used to apply 0.02 ml. of blood serum as a streak at the apex of each strip. A potential of 220 v. was applied for 2 hours. The paper strips were then spread flat (to avoid shifts in the positions of the protein bands) and dried at room temperature in a current of air. Each serum was run in triplicate, one strip being used as a "marker" for staining to locate the positions of the lipoprotein bands.

Staining

The marker strips were dried at 125° for 5 minutes then stained in Sudan Black solution for 30 minutes. They were rinsed in tap water and, finally, again dried for 5 minutes at 125°.

Elution

The lipid pattern revealed on each stained strip was divided into the two 3-cm. segments which best separated the α and β bands and which together included the total lipoprotein. Identical segments were cut from the corresponding unstained strips by aligning them with the stained strips at the points of application of the sera. Each unstained segment was folded to four thicknesses and forced into a loop of stainless-steel wire (1.04-mm.-diameter wire about 190 mm. long, looped at one end and bent so that a total of 145 mm. extends down into the tube, see Fig. 1). A further 3-cm. segment of paper from the cathode side of the strip was included as a blank correction. Four milliliters of elution solvent was placed in an ordinary 15- by 150-mm. Pyrex test tube for each paper segment. These test tubes were placed in a temperature-controlled water bath at 75° and a wire loop put in each tube so that the paper was submerged. Extraction was allowed to proceed for 10 minutes, a period demonstrated to be adequate for elution as shown by the data presented in Fig. 2. At the end of 10 minutes the volume of the chloroform-methanol solvent had been reduced to or below 3 ml. by evaporation, and solvent

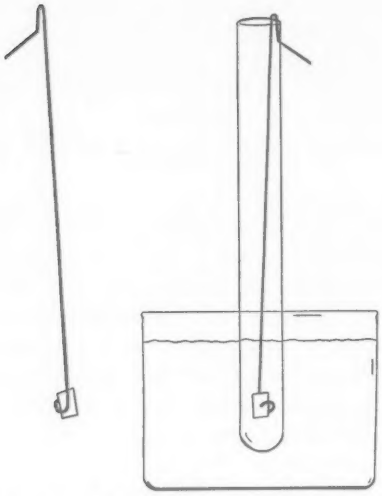


Fig. 1. Design of wire loop (made of stainless steel 1.04 mm. in diameter and 145 mm. long).

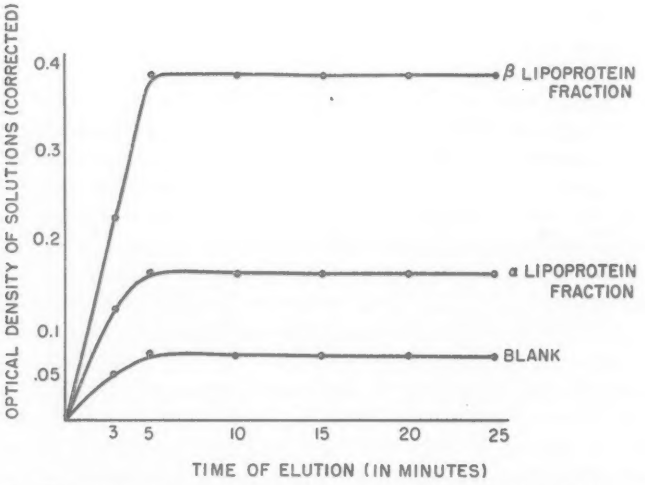


Fig. 2. The relation between time of elution of lipoprotein solution and the amount of material eluted. See text for full explanation.

was replaced to the 3-ml. mark if needed. The wire loops and paper segments were removed from the test tubes, and all solvent was evaporated off by a 20-minute heating.

Cholesterol Color Development

One milliliter of glacial acetic acid was added to the residue in each tube. Then 0.02 ml. of standard cholesterol solution was pipetted into each of two additional tubes and 0.98 ml. of glacial acetic acid added to each of these, which then served as color standards. A reagent blank was prepared by placing 1 ml. of glacial acetic acid in another tube.

After all the tubes were cooled to room temperature, 0.65 ml. of ferric chloride–sulfuric acid reagent was run into each one from a buret, allowing the reagent to run slowly down the wall of the tube. The tubes were tapped to mix the contents and were placed in a water bath at 56° ± 1° for 17 to 23 minutes. They were then removed, cooled to room temperature, and the contents transferred to matched 10- by 75-mm. round cuvets which were centrifuged briefly to remove air bubbles. Optical densities were read within 1 hour in a Coleman Junior spectrophotometer at 560 mμ, with the reagent blank set at zero optical density. The optical densities thus obtained were corrected by multiplication by a factor of 1.09 to compensate for the eluate retained in the extracted paper (see below for the method of determining this factor). Calculations were based on the value of the cholesterol standard, after correction for the elution blank.

RESULTS AND DISCUSSION

Table 1 shows the cholesterol values obtained for two sera in repetitive runs at 2-day intervals for periods up to 10 days; the consistency of these data indicates the adequate reproducibility of the method.

Table 2 records the results obtained with five serum samples by the present method compared with the values for the same sera by the method of Langan, Durrum, and Jencks (1). Agreement between measurements by the two procedures is good.

It should be noted that submersion of a paper segment for 5 minutes in the boiling solvent mixture extracts nearly all the lipoprotein (see Fig. 2). The correction factor of 1.09 applied compensated for the amount held on the paper after extraction and removal. This factor was obtained from ten accurate measurements of the weight of the elution fluid removed from a tared bottle by dipping into it a wire loop holding a folded 2.9 × 3.0 cm. filter-paper segment. Evaporation was kept minimal by recap-

Table 1. Reproducibility: Repeated Determinations of α- and β-Lipoprotein Cholesterol in Two Sera over Extended Periods of Time

Serum	*Cholesterol in mg./100 ml.*			*% α*	*Day*
	α	*β*	*Total*		
B 81	45	206	251	17.9	1
B 81	47	221	268	17.4	3
B 81	44	207	251	17.5	5
Mean	45	211	256	17.6	
BC 75	61	192	253	24.0	1
BC 75	66	194	260	25.4	3
BC 75	63	192	255	24.5	5
BC 75	61	199	259	23.3	7
BC 75	61	195	256	24.1	9
BC 75	62	192	254	23.6	11
Mean	62	194	256	24.2	

Table 2. α- and β-Lipoprotein Cholesterol of Five Sera Estimated by the Shortened Method and That of Langan, Durrum, and Jencks[a]

Serum	*Langan, Durrum and Jencks*			*Present method*		
	α	*β*	*Total*	*α*	*β*	*Total*
A	59	210	270	59	214	273
B	51	235	286	54	230	284
C	67	201	268	66	194	260
D	46	163	209	31	170	201
E	31	180	211	40	182	222

[a] All values in mg./100 ml.

ping the weighing bottle immediately on removal of the paper. The error introduced by this correction factor is relatively small, with a coefficient of variation in ten trials of 2.5 per cent, a standard deviation of 0.0085, and a standard error of the mean of 0.003.

If electrophoretic separation of the serum into α- and β-lipoprotein fractions is omitted, this procedure can be used to estimate total serum cholesterol. For this purpose, serum is applied to a paper strip and allowed to dry. The appropriate paper segment is cut out, folded, and the elution and color development made in the same way as for the electrophoretically separated lipoproteins. (Time for a determination of total cholesterol is not more than 2 hours.)

To compare the present method for measuring total serum cholesterol with that of Sperry and Webb, values for a group of 40 sera, with a wide distribution of cholesterol values, were determined by both procedures. The mean value and the range by the present method were found to be

233.1 mg./100 ml. and 135 to 348 mg./100 ml., respectively. For the Sperry and Webb procedure the mean was 240.1 mg./100 ml. and the range 152 to 338 mg./100 ml. The ratio of the two variances was found to be 1.01 and the coefficient of correlation 0.94, indicating no significant difference and good agreement between the methods.

SUMMARY

The procedure of Langan, Durrum, and Jencks (1) for the estimation of serum α- and β-lipoprotein cholesterol has been simplified, and the time required for an analysis greatly reduced.

The present method can be used for the rapid measurement of total serum cholesterol as well as for distribution between α- and β-lipoproteins.

REFERENCES

1. Langan, T. A., Durrum, E. L., and Jencks, W. P., *J. Clin. Invest.* **34**, 1427 (1955).
2. Swahn, B., *Scandinav. J. Clin. & Lab. Invest.* **5**, suppl. 9 (1953).
3. Brice, A. T., Jr., *J. Lab. & Clin. Med.* **19**, 1113 (1934).
4. Williams, F. G., Jr., Pickels, E. G., and Durrum, E. L., *Science* **121**, 829 (1955).
5. Sperry, W. M., and Webb, M., *J. Biol. Chem.* **187**, 97 (1950).

the Clinical Chemist

HONORARY MEMBERSHIP TO LINUS PAULING

The American Association of Clinical Chemists has conferred an honorary membership on Linus C. Pauling, Professor of Chemistry at the California Institute of Technology and Director of the Gaste & Crellin Laboratories at Caltech. Pauling was born in Portland, Oregon, on Feburary 28, 1901, and received a B.S. from Oregon State College and a Ph.D. from California Institute of Technology. He has also received many honorary degrees from universities both here and abroad. Besides the Nobel Prize awarded Pauling in 1954, he has been honored with the Langmuir Prize, Nichols Medal, Gibbs Medal, and Richards Medal, among others. Pauling served as President of the American Chemical Society in 1948.

The scientific interests of the recipient range from ferromagnetism and metals and alloys to the structure of proteins and the abnormalities in sickle-cell anemias, and he has made outstanding contributions in each of these fields of interest. His reputation is as great with the freshman chemistry student as it is with the family physician. The citation on honorary membership to Pauling is an understatement, as indeed any citation to him must be.

The award was presented to Professor Pauling at a meeting of the Southern California section held on March 5, 1957, in the auditorium of the Childrens Hospital in Los Angeles. The meeting was opened by William R. Bergren, program chairman, who in turn introduced Kenneth D. Johnson, chairman of the section. The formal presentation to Linus Pauling was made by Robert M. Hill, President of the Association and Professor of Biochemistry at the University of Colorado. Professor Pauling accepted honorary membership with an address on "The Structure of Water and of Aqueous Solutions." The award was accompanied by a membership scroll and the following citation:

Citation for Professor Linus Pauling

You have made extraordinary contributions to human knowledge, particularly to elucidation of the nature of the chemical bond and structure of complex substances. Equally important are the methods you employed for constructive use of the human mind. Your theories and execution of brilliant experiments contributed to understanding of proteins, immunological reactions and developing the concept of "molecular diseases." You have stimulated and inspired those working in Clinical Chemistry. Because you have dedicated yourself unremittingly to the highest traditions of the scientist and enriched the funda-

mental understanding of the chemical reactions of the human body, the American Association of Clinical Chemists chooses you for honorary membership.

NEWS FROM THE SECTIONS

Philadelphia

Intricacies and difficulties of lead determination were discussed at the forty-second scientific session of the Philadelphia section in a paper given by Fredric Rieders. The meeting was held on Tuesday, January 22, 1957, at the Physical Sciences Building of the University of Pennsylvania.

Dr. Rieders is Toxicologist to the Medical Examiner of the City of Philadelphia. He presented an ingenious technic whereby transfer of materials through the steps of digestion, extraction, and color development were so arranged as to limit the sources of contamination and loss. Many practical suggestions were offered in the outline of the method.

The forty-third scientific session was held at the Hospital of the University of Pennsylvania on February 26, 1957. The speaker was Dr. Peter Kuo, Assistant Professor of Medicine at the University of Pennsylvania, who spoke on "The Determination of Serum Lipoproteins and Clinical Applications."

Peace Paubionsky

New York

Section officers for 1957 follow:

Chairman: Charles L. Fox, Jr., *New York Medical College.*

Vice-Chairman: Edward Muntwyler, *State University of Brooklyn.*

Secretary-Treasurer: Abraham Saifer, *Jewish Chronic Disease Hospital of Brooklyn.*

Texas

The Texas section met at Parkland Memorial Hospital in Dallas for a symposium on toxicology. Among the discussants were Drs. E. L. Pratt, Kathryn Willis, Don Sutherland, Andres Goth, and M. S. Mason, all of the University of Texas Southwestern Medical School, and Capt. A. C. Dixon of the 4th Army Medical Laboratory.

At the annual business meeting held during the afternoon the following officers were elected for 1957:

Chairman: Morton F. Mason, *Southwestern Medical School, Dallas.*

Chairman-Elect and Program Chairman: Ferrin B. Moreland, *Baylor University College of Medicine, Houston.*

Secretary-Treasurer: A. C. Dixon, *4th Army Medical Laboratory, Fort Sam Houston.*

James L. Gilleland of the Veterans Administration Hospital in Houston was appointed chairman of the membership committee.

Ferrin B. Moreland

COLLOQUIUM AT BRUGGE

The Vth Colloquium of St. Jans Hospital at Brugge, Belgium, will be held from May 4–6, 1957, on "The Protides of Biological Fluids." A plenary session is planned to include, among other speakers, Antweiler (Bonn), Burtin (Paris), Franglen (London), Hannig (Munich), Huisman

(Groningen), Lontie (Leuven), Peeters (Brugge), Sanz (Geneva), Schultze (Marburg/Lahn), Seligman (Paris,) Uriel (Paris), Wunderly (Zurich).

In the afternoon of the first day there will be a round-table discussion on standardization of electrophoresis in view of its clinical application. The following day will feature a conference conducted by A. Tiselius (Uppsala) on "Electrophoresis: Past, Present and Future."

Communications related to the Colloquium should be sent to: Laboratorium, St. Jans Hospital, Brugge, Belgium.

BOOK REVIEWS

Introduction to Chemical Pharmacology

R. B. BARLOW. New York, John Wiley and Sons, Inc., 1955, 343 pp. + xiv, $6.25.

This book is concerned with the segment of chemical pharmacology that deals with the structure-action relationships of drugs, especially those affecting the nervous system.

The book starts with a discussion of biological variation of the use of statistics and of a short review of the theory of drug action. Following this are sections describing drugs with the following actions: (1) general depression of the central nervous system; (2) depression of specific centers of the central nervous system (anticonvulsants, analgesics, etc.); (3) stimulation of the central nervous system; (4) depression of peripheral nerves (local anesthetics); (5) stimulation or depression of nerve transmission across peripheral synapses (acetylcholine-like compounds; antispasmodic and mydriatic agents, ganglionic-blocking agents, neuromuscular blocking agents, anticholinesterases, adrenaline-like compounds, adrenergic blocking agents); (6) drugs that act on tissues and organs (antihistaminics, compounds acting directly on smooth muscle, drugs affecting heart muscle). An appendix gives a brief account of biochemistry, physiology, and anatomy for the reader who lacks biological background.

Accompanying each section is a detailed account of the possible modes of action of the drugs and the correlation of their structure with activity.

The book is stimulating and excellently written and should be of great value in converting chemists interested in bridging the gap between chemistry and pharmacology. However, certain drawbacks are evident to the researcher in medicinal chemistry. As the author states, the book is an outcome of a course in chemical pharmacology for students in chemistry in 1945. This may explain the author's tendency to be somewhat dogmatic concerning a number of views that were seriously considered by pharmacologists in 1945 but are no longer tenable today.

For instance, it is stated that stimulation of postganglionic sympathetic nerves releases two hormones, norepinephrine for the stimulatory, and epinephrine for the inhibitory, effects, when it is now generally considered that liberated norepinephrine is perhaps the only adrenergic mediator liberated. Again, recent evidence makes it almost impossible to accept the conception that ephedrine and amphetamine act by blocking monoamine oxidase and thereby preserving norepinephrine. The statement that thiobarbiturates have a shorter duration of action than their oxygen analogues because they are rapidly metabolized has

been disproved by a large body of work by many investigators, showing the short duration of the sulfur compounds results from their localization in body fat.

The treatment of structure-activity relationships is carefully done, with plentiful tables and structural formulas to facilitate the reading of the text. However, it seems to the reviewer that the difficulty of interpreting effects of a drug on isolated tissue (Table 83) in terms of its potential value in man should have been stressed more strongly. More surprising is the failure of the author to point out that a change in structure of a drug may decrease its activity in vivo, not by changing its inherent ability to modify body function, but by changing its physical or chemical properties so that it fails to reach the site of action in sufficient amount, by not being absorbed, by too rapid excretion or metabolism, or by failing to cross a body membrane such as the blood-brain barrier.

Laboratory of Chemical Pharmacology, BERNARD B. BRODIE
National Heart Institute, Bethesda, Md.

Ionography: Electrophoresis in Stabilized Media

H. J. MCDONALD. Chicago, The Year Book Publishers, Inc., 1955, 268 pp., $6.50.

Readers of our former publication, *The Clinical Chemist,* will recognize Prof. McDonald as the author of the excellent review series on ionography, for which both author and publication received much praise. Now, with the new technic being greatly utilized in clinical and biochemical investigations, McDonald and his collaborators have gathered those phases of investigations dealing with the technic and have organized them into a most useful text.

The book is well written and excellently organized. The first six chapters give a clear picture of the history, theory, and instrumentation. The later chapters deal with applications of the technic to biochemical substances, inorganic material, and miscellaneous investigations, such as antibiotics, alkaloids, and viruses. A most complete bibliography of 865 papers is included.

This work will make an excellent text for student instruction and is "must" reading for all who are using or plan to use this new investigative technic.

ABSTRACTS

Editor: Ellenmae Viergiver. *Contributors:* Joseph S. Annino, Gladys J. Downey, Clyde A. Dubbs, Alex Kaplan, Margaret M. Kaser, Miriam Reiner, Herbert Thompson

The estimation of magnesium in serum using titan yellow. D. W. Neill and R. A. Neely (*Royal Victoria Hospital, Belfast, Ireland*)

The titan yellow method for estimating serum magnesium has been modified by the addition of calcium to the magnesium standard solutions, and by the use of gum ghatti in place of hydroxylamine hydrochloride as a color stabilizer.—*J. Clin. Pathol.* **9,** 162 (1956). (E. V.)

Flame photometry. D. E. Brown (*Hackensack Hospital, Hackensack, N. J.*

The subject of flame photometry is discussed and descriptive information is given for 8 flame photometers in use in clinical laboratories.—*Am. J. Clin. Pathol.* **26,** 807 (1956). (H. E. T.)

Flame photometric determination of plasma calcium. G. H. Marquardt, G. M. Cummins, Jr., M. L. Phillips, and C. I. Fisher (*Chicago Wesley Memorial Hospital and Northwestern University Medical School, Chicago, Ill.*)

Using a generally accepted method for the chemical determination of calcium as a means of comparison, the flame photometric technic was at least as accurate, and less time-consuming. The instrument used was the Weichselbaum-Varney.—*Am. J. Clin. Pathol.* **26,** 1094 (1956). (H. E. T.)

Spectrophotometric titration of serum calcium and magnesium. B. Zak, W. M. Hindman, and M. Fisher (*Wayne University College of Medicine, Detroit, Mich.*)

A rapid spectrophotometric method is described for the determination of calcium and magnesium in 0.5 ml. of serum. The determinations are based on complexometric titration with ethylenediamine tetraacetic acid as the titrant and eriochrome black T as indicator. Recovery and control studies showed good agreement between observed and theoretical values.—*Am. J. Clin. Pathol.* **26,** 1081 (1956). (H. E. T.)

A rapid ultraviolet spectrophotometric method for the detection, estimation and identification of barbiturates in biological material. P. M. G. Broughton (*Radcliffe Infirmary, Oxford, England*)

A rapid method for the measurement of as little as 10 μg. of barbiturates per ml. of blood, urine, gastric contents or tissue homogenates is described. The

barbiturate is extracted from the biologic fluid with chloroform, transferred to an aqueous medium by shaking with dilute alkali, and the optical density measured at *p*H 13.4 and at *p*H 10 from 228 to 265 mμ. *Biochem. J.* **63,** 207 (1956). (A. K.)

Photometric determination of serum cholinesterase activity. W. T. Caraway (*Rhode Island Hospital, Providence, R. I.*)

Liberation of acetic acid from acetyl choline by serum cholinesterase was analyzed photometrically by measurement of the decrease of absorbance of phenol red indicator. Variations in initial absorbance were nullified by a calibration based on the ratio of the final to the initial absorbance.—*Am. J. Clin. Pathol.* **26,** 945 (1956). (H. E. T.)

An improved procedure for the determination of hydrocortisone in human plasma. R. H. Silber and R. D. Busch (*Merck Institute for Therapeutic Research, Rahway, N. J.*)

A modification of the Silber-Porter procedure for the determination of hydrocortisone in plasma has been described which has greater sensitivity and avoids the addition of standard to the sample as well as the use of standards in plasma.—*J. Clin. Endocrinol. and Metabolism* **16,** 1333 (1956). (M. R.)

Further experience with the toad method of testing for chorionic gonadotropin in normal and in disturbed pregnancy. E. H. Hon and J. M. Morris (*Yale University, New Haven, Conn.*)

The male toad, *Bufo marinus,* appears to be a satisfactory animal for use in tests to establish the diagnosis of pregnancy and for quantitative estimation of urinary chorionic gonadotropin in the disturbances of pregnancy. Availability, minimal seasonal variation in sensitivity, and easy husbandry suggest that it is more suitable as a test animal than the batrachians previously studied. Using this toad in the assays, studies of the quantitative estimation of urinary chorionic gonadotropin confirm its prognostic value in disturbed pregnancies.—*J. Clin. Endocrinol. and Metabolism* **16,** 1354 (1956). (M. R.)

Aldosterone excretion in healthy persons. E. H. Venning, I. Dyrenfurth, and C. C. P. Giroud (*McGill University Clinic, Montreal, Canada*)

The excretion of aldosterone in the urine of healthy adult subjects has been determined by means of a bioassay technic based on the excretion of sodium in adrenalectomized rats. The mean of 50 determinations in 12 normal men was 3.2 ± 1.4 μg. per 24 hours. There was considerable variation from day to day in the same individual. In 6 of 9 subjects there was a diurnal rhythm. In 5 sub-

jects an increased excretion of aldosterone was associated with a stressful situation. *J. Clin. Endocrinol. and Metabolism* **16,** 1326 (1956). (M. R.)

Serum glutamic pyruvic transaminase in cardiac and hepatic disease. F. Wroblewski and J. S. LaDue (*Sloan-Kettering Institute, New York, N. Y.*)

Glutamic pyruvic transaminase is present in higher concentration in liver than in heart muscle, whereas glutamic oxaloacetic transaminase occurs in high concentration in both tissues. Data are given which show that serum glutamic pyruvic transaminase (SGP-T) is elevated in hepatitis and is a somewhat more sensitive indicator of hepatocellular injury than is the serum glutamic oxaloacetic transaminase (SGO-T), but it is not increased in myocardial infarction. The SGP-T concentration is determined by the same procedure as for SGO-T except that alanine is substituted for aspartate in the reaction mixture.

Analysis of sera from 260 normal subjects gave values of 16 ± 9 units/ml./minute. In a study of 40 patients with acute hepatic disease the SGP-T concentration was elevated from 2 to 1000 times the normal and was somewhat greater than the SGO-T activity. Both SGP-T and SGO-T were increased in cirrhosis in relapse but the increases in SGO-T were greater. Infiltration of the liver with metastatic cancer or leukemic or lymphomatous disease may increase the SGO-T but did not affect the SGP-T. SGP-T was not increased in myocardial infarction or in a number of other conditions.—*Proc. Soc. Exp. Biol. Med.* **91,** 569 (1956). (M. M. K.)

The thymol test: A study of factors affecting its accuracy and a description of a modified technic. J. G. Reinhold and V. L. Yonan (*Hospital of University of Pennsylvania, Philadelphia, Pa.*)

Slight variations in the method of preparing thymol-barbital reagent may affect considerably the measurement of thymol turbidity. This has led to difficulties in distinguishing the relatively small but clinically significant differences that may exist between normal and abnormal sera.

A modification of Maclagan's procedure is described for the preparation of the thymol-barbital reagent which gives greater stability and improved reproducibility.—*Am. J. Clin. Pathol.* **26,** 669 (1956). (H. E. T.)

A new method for the determination of serum iron-binding capacity. T. Peters, T. J. Giovanniello, L. Apt, and J. F. Ross (*Veterans Administration Hospital, Boston, Mass.*)

A simple chemical procedure applicable to fresh or stored sera and to lipemic and icteric specimens is presented for the determination of total serum iron-binding capacity. The serum is first saturated with ferric ammonium citrate. Iron which is not bound by serum proteins is removed by the addition of an ion

exchange resin and the supernatant is then analyzed for iron.—*J. Lab. Clin. Med.* **48,** 274 (1956). (G. J. D.)

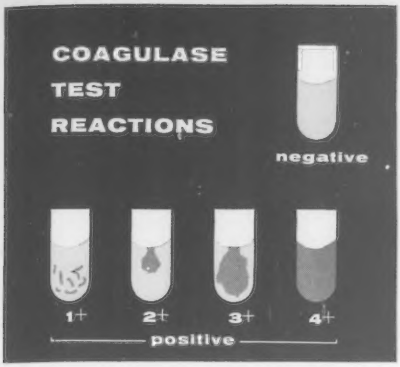
A simple improved method for the determination of serum iron. T. Peters, T. J. Giovanniello, L. Apt, and J. F. Ross (*Veterans Administration Hospital, Boston, Mass.*)

A modification of the Barkan and Walker procedure for the determination of iron is presented which utilizes 4-7-diphenyl-1,10-phenanthroline for the color development. The precision of the method is about 1 μg. per 100 ml. and does not involve heating, extraction with organic solvents, daily preparation of reagents, or multiple centrifugations. The method is applicable to lipemic and icteric specimens.—*J. Lab. Clin. Med.* **48,** 280 (1956). (G. J. D.)

A systematic evaluation of the Nelson-Samuels plasma 17-OH-corticosteroid method. C. T. Harwood and J. W. Mason (*Walter Reed Army Medical Center, Washington, D. C.*)

The Nelson-Samuels method is difficult but very reliable for the measurement of minute amounts of 17-OH-corticosteroids in plasma. The preparation of materials is critical, particularly the washing and activation of the Florisil.

The precision of the method at the 1 μg. level is ±0.23 μg., with the standard deviation approximately constant up to 6 μg.—*J. Clin. Endocrinol. and Metabolism* **16,** 790 (1956). (M. R.)



REACTIONS AND REAGENTS

- *standard serum for standardized results*
- *pinpointing staph infections*
- *purified reagent for coagulation research*

A Plea for Unity

Participants at a recent International Congress of Clinical Chemistry agreed: Standardized data from clinical laboratories throughout the world would be invaluable. The International Biochemical Trial 1954 has shown that laboratories can duplicate their own results more readily than they can determine the absolute value of constituents in an unknown test serum. Comparing the patient's serum with a known serum would eliminate this source of error immediately.[1] Versatol, the only *control* in whole human serum which is also a *standard*, solves this problem. It contains known normal amounts of protein-bound iodine, total nitrogen, total protein, nonprotein nitrogen, urea nitrogen, creatinine, glucose, sodium, potassium, calcium, chloride and phosphate. Each determination is performed in the presence of all the complex substances in the patient's blood. Because Versatol is precisely standardized by special techniques, it can take the place of individual aqueous standards wherever a standard is called for in your procedure. Errors caused by aqueous solutions are eliminated. Versatol is ready for use by just adding distilled water. We will be glad to send you more detailed information.

1. Wootton, I. D. P.: Paper presented at International Congress of Clinical Chemistry, New York, Sept. 9, 1956.

standard and control for 12 blood chemistry tests

VERSATOL
TRADEMARK

How Nurses Become Infectors

The "nurse nasal index" measures the extent to which coagulase-positive staphylococci colonize hospitals.[2] To compile this "index," nasal passages of student nurses were examined for potentially pathogenic organisms. A "typing scheme" was then drawn up based on staphylococcal phage typing and antibiotic sensitivity. The survey showed that cross-infection was possible only after ward duty was begun. This combined information provides an index which may prove valuable in controlling hospital-spread infections. For laboratories interested in identifying pathogenic staphylococci, Diagnostic Plasma Warner-Chilcott is a convenient source of fresh human plasma. Positive coagulase reactions with rabbit and other nonhuman substrates are not completely valid as an index of pathogenicity for humans.[3] With Diagnostic Plasma, results are identical to those obtained with citrated freshly drawn plasma. Coagulation is usually observed within an hour[4]—the longer incubation time necessary with blood bank plasma allows the fibrinolysin-producing strains to lyse the clot, causing "false negatives."

2. Brodie, J.; Sommerville, T., and Wilson, S. G. F.: Brit. M. J. *1*:667 (Mar. 24) 1956. 3. Conference on Staphylococcal Infections, New York Academy of Sciences, Feb. 16, 1956. 4. Boyd, H.: Am. J. M. Technol. *22*:232 (July-Aug.) 1956.

for prothrombin time normals and coagulase tests

DIAGNOSTIC PLASMA
WARNER-CHILCOTT

What's in a Name

Call it what you will, each blood clotting factor fulfills its own specific functions. However, one blood factor has been discovered independently by fifteen different investigators and given as many names.[5] Such complex research requires highly purified reagents. Fibrinogen Warner-Chilcott facilitates blood coagulation studies because it is free of citrate, fibrinolysins, and other coagulation factors. The tedious, time-consuming task of purification is eliminated. Fibrinogen Warner-Chilcott is suitable as a fibrinogen standard, or for the serum prothrombin consumption test, two-stage prothrombin time, antithrombin titer, and any other phase of research requiring fibrinogen. By simply adding distilled water to the lyophilized bovine fibrinogen, a solution is formed containing 300 mg.% clottable protein in physiological saline. No further preparation is necessary.

5. Seegers, W. H.: J. Michigan M. Soc. *55*:272 (Mar.) 1956.

for prothrombin consumption tests and coagulation research

FIBRINOGEN
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MICROTOME KNIFE
Sharpener

NEW!
Improved Model

The RSCo Model 2200 MICROTOME KNIFE SHARPENER is an improved instrument based upon the design of Professor J. A. Long of the University of California at Berkeley. The Long Sharpener has proved itself over a period of years as the most desirable for routine use in keeping microtome knives in excellent condition for the most exacting of sectioning requirements. The major features of the basic design that have led to its success are the use of a soap solution in which the abrasive is suspended and the manual method of sharpening in which the operator's sense of "feel" is involved. No machine can substitute for this "feel" in producing the finest of edges. The liquid abrasive suspension acts as a coolant and cleaner, thus preventing burning and chipping of the edge.

The superiority of this model over earlier instruments lies in its design features which lead to less complex operation, longer life, and simplified maintenance.

The knife guide and trough are cast into a single piece forming the base. A drain spout permits easy cleaning without moving the base.

The spindle housing is easily removable from the base for cleaning and for service. Adjustment for proper knife bevel angle is accomplished by a micrometer dial reading to 0.001." The sliding ways for the housing (allowing variable positioning for different bevel angles) are ground steel surfaces, giving smooth action and rigid mounting. A locking knob holds the housing in its adjusted position.

The glass wheel and the spindle assembly are mounted integrally with the motor in a single housing. Two self-aligning double-row ball bearings insure perfect spindle alignment. Axial spring loading on the bearings insures years of service without attention. One of the most important features is the wheel rotation speed of 850 rpm. This speed has been found to be optimum for sharpening; higher speeds cause burning of the knife edge.

The wheel dresser for the sharpener uses a diamond impregnated stone. The dresser can be rented or purchased.

DESCRIPTION

Overall dimensions: 19" width x 14" depth x 8" height
Net weight: 24 lbs.
Wheel speed: 850 rpm
Wheel dimensions: 5" diam. x ½" thick
Power requirements: 115 volts, 60 cycles
Equipment Supplied:
Sharpener complete with 6 lbs. Levigated Alumina
Wheel Wrench
Splash Guard
Instruction Book
Power Cord: 6 ft. long with 3 prong ground type plug and adapter to 2 prong outlet

PRICE

Cat. No. V-75010 Microtome Knife Sharpener
Complete, as above . . $250.00

ACCESSORIES

Cat. No. V-75011 Diamond Wheel Dresser $50.00

Cat. No. V-75012 Levigated Alumina 6 lb. $4.00

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